

PRACTICAL BOTANY

A.H. REGINALD BULLER

PRACTICAL BOTANY

By the same Author

RESEARCHES ON FUNGI

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FUNGI OF MANITOBA

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PRACTICAL BOTANY

AN ELEMENTARY COURSE IN THE
GENERAL MORPHOLOGY AND PHYSIOLOGY OF PLANTS
ESPECIALLY ADAPTED FOR THE REQUIREMENTS
OF STUDENTS AT THE UNIVERSITY OF
MANITOBA

BY

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WITH TWO DIAGRAMS IN THE TEXT

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PUBLISHED FOR THE UNIVERSITY OF MANITOBA BY
LONGMANS, GREEN AND CO.
LONDON • NEW YORK • TORONTO

1929

LONGMANS, GREEN AND CO. LTD.

39 PATERNOSTER ROW, LONDON, E.C.4
 6 OLD COURT HOUSE STREET, CALCUTTA
 53 NICOL ROAD, BOMBAY
 167 MOUNT ROAD, MADRAS

LONGMANS, GREEN AND CO.

55 FIFTH AVENUE, NEW YORK
 221 EAST 20TH STREET, CHICAGO
 TREMONT TEMPLE, BOSTON
 128-132 UNIVERSITY AVENUE, TORONTO

PREFACE

This book owes its origin not to the need of meeting the requirements of any examination or to the hope of financial reward, but solely to the desire of the author to assist his own pupils in studying the plant material supplied to them for practical work in connection with their course in Elementary Botany.

It was the intention of the author to have this *Practical Botany* published privately and sold only by the Book-room of the University of Manitoba ; but, at the request of several friends, after the proofs had come to hand, he decided to ask Messrs. Longmans, Green and Company to give the general botanical public access to it in the usual way. If what has been set down as a result of twenty-five years' experience of teaching at the University of Manitoba should prove of any assistance to other teachers and their students, the author will be gratified.

This *Practical Botany* was first produced in the form of Schedules in 1926-1927, and in that and the two following years every student taking practical work in Elementary Botany at the University of Manitoba was given a multigraphed copy of a Schedule weekly throughout the session. The number of students now requiring Schedules has grown to upwards of two hundred, and it is on this account that the author has thought it worth while to publish the Schedules in book form.

Each chapter of the book provides for a single lesson of about two hours' duration, and the twenty-four chapters are to be worked through in succession, one each week throughout twenty-four weeks of the University session.

The absence of botanical illustrations from the book is an advantage in that it compels the student to study and to endeavour to represent what he actually sees and not the drawings of others.

More stress than usual has been placed on mastering the

structure and working of the microscope, as it is the author's belief that a student, from the first, should learn to understand this important instrument and not be content with using it merely by rule of thumb.

The types, such as *Protococcus*, the Yeast Plant, Moulds, etc., are introduced in successive weeks from the thirteenth week onwards. This arrangement serves to diversify the work, to keep up the interest in the course, and to prevent the lower organisms from being hastily or insufficiently dealt with toward the end of the University session.

In choosing objects for study, the author has taken into account the limited number of hours devoted to class work, the climatic conditions and geographical position of Winnipeg, and the necessity of supplying a large number of students simultaneously with the required materials.

The text is written in such a way as to enable students to work through each lesson with a minimum of oral directions as to technique and the nature of the observations which it is intended they should make. At the end of the book, as a means of reference, a brief classification of the Plant Kingdom has been provided.

The lecture course in Elementary Botany at the University of Manitoba consists of about fifty lectures, two being given each week throughout the University session. These lectures are taken by those intending to proceed in Arts, Science, Medicine, or Pharmacy. The practical work outlined in this book is designed to accompany and to supplement the lecture course. Those intending to proceed to Medicine and Pharmacy take all the practical work, while those intending to proceed in Arts and Science take a smaller amount of practical work, as such students study Botany as a half-subject, the other half-subject usually being Zoology or Geology.

I desire to thank my colleagues, Assistant-Professor H. F. Roberts and Mr. C. W. Lowe, for useful suggestions in regard to the text, and Mr. W. B. Grove, of Birmingham, England, for help in the final revision of the proofs.

May this little book be helpful to those for whom it is intended and serve to extend and deepen their interest in the Plant Kingdom.

A. H. REGINALD BULLER.

THE UNIVERSITY OF MANITOBA,
September 15, 1929.

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PRACTICAL BOTANY

GENERAL INSTRUCTIONS FOR STUDENTS IN COURSES II AND IIA

I. EQUIPMENT

Each student must provide himself or herself with the following :

- (1) A **drawing-book** with a stiff black cover, containing blank paper. It is on sale at the University book-room.
- (2) **Pencils**. One should be an HH, suitable for making clear outlines for drawings, and another an HB, suitable for shading.
- (3) An **india-rubber**.
- (4) A **pen-knife** or **scalpel**. This is required for sharpening pencils and for various other purposes.

Razor. In addition to a drawing-book, pencils, india-rubber, and a knife, students proceeding to medicine and pharmacy must provide themselves with a **hollow-ground razor** having a folding handle. The razor will be required for making hand-sections of seeds, stems, etc., on or about the sixth day of the practical work.

II. THE RECORD OF YOUR PRACTICAL WORK

You are required to keep a complete record of your practical work in your drawing-book. The record will consist of your own notes, drawings, and answers to questions.

The number of each chapter should be written at the top of each week's notes and drawings.

The notes and drawings are to be entered on one side only of each page.

All your drawings and other work (naming of drawings and labelling their parts, making of notes, etc.) must be done in **pencil** (not ink). All drawings must be labelled. In labelling drawings, the names are to be written **horizontally** and in **long-hand** (no printing).

Your **name**, **class**, and **seat-number** must be clearly indicated on the **outside** of the front cover of your drawing-book.

Your drawing-book will be examined from time to time, and your record will be taken into account in the practical examination which will be held at the end of the session.

III. THE MICROSCOPE

A compound microscope will be provided for each student. You will be responsible for keeping it undamaged and clean.

At the end of a lesson, the microscope must be left with the low-power objective turned on and the slide removed from the stage.

CHAPTER I

SEEDS AND THEIR GERMINATION

The following botanical material will be supplied :

- (1) Dry Pea seeds of more than one variety ;
- (2) Pea fruits ;
- (3) Pea seeds, soaked in water for 24 hours ;
- (4) Windsor Bean fruits containing seeds ;
- (5) Windsor Bean seeds, soaked in water for 24 hours ;
- (6) Scarlet Runner seeds, soaked in water for 24 hours ;
- (7) Germinating Pea seeds or Pea seedlings.

1. THE GARDEN PEA—*Pisum sativum*

The Pea belongs to the great natural family of Flowering Plants called the **Leguminosae**, all the species of which have a fruit known as a **legume** or pod. There are many varieties of the Garden Pea : some are tall (6 feet), others dwarf (1-1.5 feet) ; in some the seeds are smooth, in others wrinkled ; in some the seeds are green, in others yellow. What kinds of seed have been

supplied to you? Make a list of the kinds in your book. The crossing of different varieties of the Garden Pea by Gregor Mendel (1865) led to a great advance in our knowledge of heredity.

(a) Examine the Pea fruit (legume) supplied to you. It developed from the female part of a flower. Note its **fruit-stalk** (once the flower-stalk) and the **calyx** (made up of greenish sepals, still more or less perfect at the top of the flower-stalk). The fruit-wall is known as the **pericarp**. Note that the pericarp is thin and green. Under natural conditions, when the fruit is ripe and ready to liberate its seeds, it opens up by splitting into two halves or **valves** (it dehisces along an upper or ventral suture and along a lower or dorsal suture).

Open the fruit by pulling apart the two halves or valves beginning at the fruit-apex (opposite end to fruit-stalk). Spread out the open fruit so that the two halves are more or less horizontal, with the seeds hanging downwards as they did under natural conditions.

Make a **drawing** of the open fruit showing the seeds. All drawings are to be made in pencil—not ink. An india-rubber is essential. Pencils should be an HB (for drawing) and an HH (hard, for lining in), and you should have a pen-knife or scalpel for sharpening them. Good drawings cannot be made with blunt pencils. Write above your drawing: **Open Fruit (legume) of a Pea**, and write at the side the names of the parts (**pericarp, seeds**), and indicate which parts are meant by means of lines passing to them. The names are to be written **horizontally** and in **long-hand** (no printing). The present drawing may be made **natural size**, but it is often advisable to make a drawing twice or several times the natural size. It is a mistake to make drawings on too small a scale. You must use your best judgment in this matter.

(b) Examine an individual seed. This has been soaked in water for 24 hours, so that it is now much swollen and about to germinate. It is covered by a thin tough seed-coat, known as the **testa**. Note on the exterior of the testa: (1) the scar or **hilum** left when the seed was broken away from the fruit; (2) the position of the pointed hidden **radicle**, indicated by a somewhat wedge-shaped bulge, and (3) the **micropyle**, a minute round perforation through the testa between the radicular bulge and the hilum. Possibly, if you squeeze the seed slightly, water may pass in and out through the micropyle.

Sketch the interior of a Pea seed three times the natural size

and show the parts just described. The radicular bulge should point **downwards**. Write above your drawing what the drawing is meant to represent, e.g. **Exterior View of a Pea Seed**, and name each part (**testa**, **hilum**, **bulge of radicle**, **micropyle**) as before in long-hand.

(c) Remove the testa from a seed and, as you do so, note that the radicle, which now comes into view, is directed toward the micropyle and hilum. Note the micropyle and hilum in the removed testa (hold it up to the light). After the removal of the testa, an **embryo** (a rudimentary Pea plant) remains. Examine the embryo very carefully. Open it out into its two halves, if possible without breaking it, and make out its parts. It consists of : (1) a **radicle** (rudimentary root), a smooth pointed structure ; (2) a **plumule** (rudimentary shoot, i.e. a stem bearing leaves) ; and (3) two **cotyledons**, hemispherical structures attached to the main axis of the embryo between the plumule and radicle, one on each side of the axis. The cotyledons, although not resembling ordinary foliage leaves, are nevertheless **leaves** ; but they are highly specialised, each being very fleshy, hemispherical in form, and containing a large amount of reserve food materials (starch and aleurone grains) which will be studied with the microscope later on in the session. It is the cotyledons, as **food reservoirs**, which make peas so valuable for human food.

Make a **sketch** of the Pea embryo, three times the natural size, showing the **plumule**, **radicle**, and the **two cotyledons** (their flat side in view). The radicle in the drawing should point exactly **downwards**. Name each part. Remember to write each name horizontally in long-hand.

Why is the cotyledon of a Pea embryo flat on one side ?

2. THE WINDSOR OR BROAD BEAN—*Vicia faba*

The Windsor Bean, like the Pea, belongs to the Leguminosae, and its fruit is a legume.

(a) Examine the Bean fruit (legume) supplied. Open it out carefully, and compare it with the fruit of the Pea. Again note the mode of attachment of the seeds to the pericarp.

(b) Examine an individual Bean seed, following the same directions as those given for the Pea seed. Has the seed of a Windsor Bean essentially the same structure as that of a Pea ?

Sketch (1) the exterior of a seed with the seed held so that the

bulge of the radicle looks downwards, and (2) the embryo showing the **plumule**, the **radicle**, and the **two cotyledons**. Use your own judgment as to the scale of these drawings.

3. THE SCARLET RUNNER—*Phaseolus multiflorus*

This is yet another species of Leguminosae. It is used chiefly for ornamental purposes (its flowers are a brilliant scarlet), but its seeds are sometimes eaten. It is closely related to the Kidney Bean (*Phaseolus vulgaris*), the green pods of which are used for food.

Compare the seed of the Scarlet Runner with that of a Pea or of a Windsor Bean.

4. SUMMARY OF OBSERVATIONS ON THE SEEDS EXAMINED

Is the following summary justified by your observations?—The Pea seed and the two Bean seeds examined are fundamentally alike in structure, for each consists only of an **embryo** (a rudimentary plant) enclosed and protected by a **testa** (a seed-coat). In each seed the embryo consists of a **radicle**, a **plumule**, and **two fleshy cotyledons**. These specialised leaves of the embryo contain reserve food materials.

5. GERMINATING SEEDS OF THE PEA

In the germinating seeds or in the seedlings supplied make out the parts already noted as being present in the embryo of an ungerminated Pea seed.

Is the plumule developing into a **shoot** (a stem bearing the foliage-leaves)? Is the radicle developing into the **primary root** of a seedling? Note the two cotyledons still held in by the testa. **Remove the testa** so that you may examine the cotyledons more easily. Their thick white stalks can now be readily observed.

Under natural conditions the cotyledons remain beneath the soil. They never develop into green foliage-leaves; but, instead, they give up their stored food materials to the young shoot and root, thus enabling these organs to grow in length, and then they wither, die, and decay.

Sketch one or more germinating seeds or seedlings with the

radicle looking downwards. Draw your specimens either natural size or, if you prefer, larger. As before, write above your drawing what the drawing is supposed to represent, and name each part.

CHAPTER II

SEEDS AND THEIR GERMINATION (*continued*)

The following botanical material will be supplied :

- (1) An Almond seed, with the testa loosened by putting the seeds in hot water ;
- (2) A Pea-nut fruit enclosing seeds, not roasted ;
- (3) A Castor Oil seed ;
- (4) A Coffee seed, soaked in hot water for 3 hours ;
- (5) A grain of Indian Corn, soaked in water for 24 hours ;
- (6) A grain of Wheat, soaked in water for 12 hours.

1. THE ALMOND SEED—*Prunus amygdalus*

The Almond is included in the great natural family **Rosaceae**, and it belongs to the same genus as the Peach, Plum, Apricot, and Cherry. The fruit in the genus **Prunus** has a pericarp (fruit-coat) exhibiting three distinct layers : (1) an **epicarp** (skin), (2) a **mesocarp** (fleshy), and (3) an **endocarp** (stony). The Almond nut, like a Peach stone or Cherry stone, is a seed enclosed by a stony endocarp. The mesocarp of the Almond fruit is leathery, instead of fleshy as in the Peach and Cherry ; and, when the Almond fruit is ripe, the epicarp and mesocarp split open (dehisce) and let the endocarp enclosing the seed fall out. The Almond tree grows wild in Turkestan and central Asia, and it has two varieties, one with sweet seeds (those we eat) and one with bitter seeds. The bitter seeds contain a glucoside called **amygdalin** and also an enzyme, **emulsin**, which hydrolyses amygdalin into hydrochloric acid, benzoic aldehyde (oil of bitter almonds), and glucose.

Examine the seed provided. Note its brown **testa** (seed-coat). The testa, under natural conditions, adheres closely to the rest of the seed and cannot be removed by the fingers. The seeds supplied to you have been put in very hot water for a few minutes,

with the result that the testa is now quite loose. Remove the **testa** and examine the **embryo** (rudimentary plant) which now comes into view. Does the embryo contain any chlorophyll? Does the embryo consist of a **plumule**, a **radicle**, and **two cotyledons**? Is the radicle at the blunt end or at the pointed end of the embryo? Record your answers in your book.

Sketch one of the cotyledons together with the plumule and radicle. As before, make the radicle point downwards, describe your drawing, and name its parts. Make the drawing either natural size or larger. In this matter use your own judgment.

2. THE PEA-NUT—*Arachis hypogaea*

This species belongs to the **Leguminosae**. Its flowers are produced above-ground, like those of a Pea; but, when the fruits are ripening, the fruit-stalk grows downwards and pushes the young fruit (**legume** or pod) into the soil, and the fruit ripens (the pericarp becoming hard and dry) **underground**. Hence the specific name **hypogaea** (from **hypo** = below, and **ge** = the earth). All pea-nuts are therefore obtained by digging. Pea-nuts are largely grown in sandy soil in Virginia and other southern States of the U.S.A.

Examine the fruit (legume) supplied to you. Note the remains of the elongated **flower-stalk** at one end (or the scar left behind, if the stalk is no longer present), and the curious ribs (vascular bundles) in the **pericarp**. Break open the pericarp (it splits as in the Pea along its dorsal and ventral sutures), and note the seeds lying within its cavity. How many seeds are there in a fruit?

Sketch one half of the open fruit with the seeds lying within.

Remove a seed, take off its thin, brown, papery **testa** and examine the **embryo**. Does the embryo resemble more nearly that of a Pea or that of an Almond? Record your answer.

3. THE CASTOR OIL SEED—*Ricinus communis*

This seed is often referred to as a **bean**, but it is quite different in structure from the true beans already studied. The Castor Oil plant belongs to the natural family **Euphorbiaceae**, which also includes a tree called **Hevea brasiliensis**, the most famous source of rubber.

Do not eat any part of a raw Castor Oil seed, as the seed contains

a **poisonous toxalbumin** called **ricin**. Castor Oil is extracted from Castor Oil seeds (from the oily endosperm); but, before it is used medicinally, the ricin is removed from it.

Examine the seed provided. Note that it is covered by a very hard shelly **testa** characterised by peculiar markings. At one end is a spongy structure (not present in most other seeds) called the **caruncle**. This absorbs water readily, and the water passes from it through a minute perforation in the testa (the **micropyle**) to the radicle which is at present hidden inside the seed. On one side of the caruncle is a not very clearly defined scar or **hilum**, indicating where the seed was attached to the pericarp (fruit-case).

Sketch the seed so as to show its external appearance. Let the caruncle end of the seed (and therefore the radicle end) look downwards on your page.

Now, with the help of a pen-knife or scalpel, remove the **testa**. In doing so, note which end was close to the caruncle. The interior of the seed is covered by a second seed-coat of silky texture often called the **tegmen**. With a knife, cut off the end of the seed which is away from the caruncle, the cut to be made transversely about 2-3 mm. from the extreme end of the seed. You should now be able to see an **air-space** in the middle of the seed. This space is between the two thin cotyledons of the embryo. Now, along the median line indicated by the air-space, cut the seed about half-way down, and then gently press the two halves of the seed apart. Examine the exposed faces of the two halves of the seed. On one you will find the **radicle** (which was pointing toward the micropyle and caruncle), the **plumule** (a mere point, scarcely developed), and **one** of the two **cotyledons**. On the other half you will find the **other cotyledon**; this cotyledon broke away from the rest of the embryo to which it was attached just at the top of the radicle. Carefully observe that each of the two cotyledons is an extremely **thin colourless leaf** with a **mid-rib** and **lateral veins**. These cotyledons do not contain the reserve food materials (they are too thin for that), and these materials (oil, aleurone grains, etc.) are all stored in the **endosperm**, a thick oily mass of tissue which completely **surrounds and encloses the embryo**. Note the massive white oily endosperm and that each cotyledon is spread out on it in the air-space in the middle of the seed. When the seed germinates, the very thin cotyledons absorb food materials directly from the

massive endosperm, and convey them to the radicle and plumule to be used for the growth of the embryo into a seedling.

Sketch one-half of the seed so as to show : (1) the **embryo** with one of its cotyledons, the **radicle**, and the very rudimentary **plumule** (a mere point) if you can make it out, and (2) the **endosperm** extending around the outside of the cotyledon. Also indicate on the outside of your drawing what the drawing is meant to represent, and name each part in long-hand with the names written horizontally. How does the Castor Oil seed differ from a Pea seed ? Record your answer.

4. THE COFFEE SEED—*Coffea arabica*

The Coffee plant is a native of Abyssinia, and the word **coffee** is derived from an Arabic word **Qahwa**, which means **wine**. Coffee is largely grown in Brazil and Central America, and also in Java. The U.S.A. is not warm enough to grow the plant as a crop. The Coffee seed is usually referred to in commerce as the **Coffee Bean**, but the Coffee plant belongs not to the Leguminosae but to the **Rubiaceae**. The fruit of the coffee is something like that of a cherry, but it contains two seeds instead of one. The pulp and hard endocarp are removed mechanically and then the seeds are left behind.

Examine the seed supplied. One side is **flattened**. The reason for this is that there were two seeds in the fruit and they pressed against one another as they ripened. Note the groove on the flat side. The thin papery **testa** was removed in preparing the seeds for market, as it has no economic value. All that you now have before you is the massive horny **endosperm** which encloses and completely hides a very small **embryo**. With a sharp scalpel or pen-knife slice away from the rounded side of the softened endosperm a succession of thin slices, and thus **find the white embryo**. Do your best not to injure the **embryo**. Remove it from the endosperm intact. Note that the isolated embryo consists of (1) an elongated pointed **radicle**, and (2) **two** small rounded **cotyledons** closely appressed to one another. Separate the cotyledons slightly with a needle or knife and look for (3) the **plumule** between them. It may be too rudimentary to be observed. Is it ?

How does the Coffee seed resemble the Castor Oil seed and differ from the Pea seed ? Record your answer.

5. THE GRAIN OF INDIAN CORN (MAIZE)—*Zea Mays*

Indian Corn belongs to the great grass family known as the **Gramineae**, and it has larger grains than any other grass species. Its wild ancestry is unknown, but it was first grown by the American Indians, who must have selected it from some wild grass species. There are six principal races of Corn: Pop-corn, Sweet corn, Starch corn, Flint, Dent, and Podded corn. The **tassel** at the top of a corn plant consists of male flowers only. These supply pollen which falls on to the long stigmas (silk) of the corn ears. An **ear** consists of female flowers only. Each female flower has an ovary containing one ovule. The ovary wall develops into a **pericarp** and the ovule matures into a **seed**. The **pericarp** (fruit-coat) and the **testa** (seed-coat) become fused together. A ripe ear of Corn is then a cob bearing a large number of **fruits**. Remember that a **grain is not a seed but a fruit containing a seed**. This is true for cereal grains of all kinds.

Examine a soaked grain of Corn. Note its stalk (fruit-stalk) at the pointed end and that the grain is flattened where it pressed against adjacent grains on the cob. On one flat side you will perceive an **oval whitish area**. This marks the position of the **embryo** which lies beneath the membrane made up of the fused pericarp and testa. The rest of the grain under the external membrane consists entirely of **endosperm**, a tissue outside of the embryo, in which the reserve food materials (chiefly starch) are stored up. Make a **drawing** showing the parts just described.

Remove the pericarp and testa (the external membrane) with a pen-knife or scalpel. Now there remain only the **endosperm** and **embryo**. Separate the embryo from the endosperm in contact with it. Examine the embryo and make out the single broad shield-like **cotyledon** (scutellum), the **radicle**, and the **plumule**. If necessary, dissect the embryo until its parts come into view.

Take another intact grain, lay it flat on the table, and bisect it, letting your knife or scalpel slice it in such a way as to cut the endosperm and embryo lengthwise into two exactly equal halves. The embryo will be cut down its middle. Now examine the cut surface very carefully. Note that you can see: (1) the **endosperm**, chiefly white in colour (some of it may be horny and glassy) occupying the upper two-thirds of the section, (2) the **embryo**, occupying the lower third of the section, and (3) the

combined pericarp and testa forming a thin membrane around the whole grain. With a little trouble, using a needle as a help, you should be able clearly to distinguish in the embryo : (1) the broad obliquely situated **cotyledon** (scutellum) with its outer surface applied to the endosperm, (2) the **plumule**, and (3) the **radicle**. The cotyledon, when the grain germinates, acts as an absorbent organ and absorbs food material from the endosperm through its broad outer surface.

Sketch the section just described. Make the drawing at least three times the natural size, and name the parts in the usual way.

6. THE WHEAT GRAIN—*Triticum sativum*

A grain of Wheat, like a grain of Corn, is not simply a seed, but it is a seed invested with a fruit-coat or pericarp. It is therefore a fruit. It is comparable to the pod of a pea enclosing its seeds.

The grain of Wheat supplied, like the grain of Corn, has been soaked in water and is about to germinate.

Examine the grain carefully. Note that it is oval in form, has hairs at one end, is grooved on one side, and is rounded on the other side. Find the position of the **embryo** at the base of the rounded side. The embryo is hidden beneath the external membrane made up of the combined **pericarp and testa**. The wheat grain, as is obvious, chiefly consists of **endosperm**, the embryo occupying but a small part of the volume of the whole grain.

Sketch a grain so as to show (1) its grooved side, and (2) its rounded side with the embryo at its base. Make your drawing several times the natural size.

Bisect the grain down the middle of the rounded side so as to divide it into two halves, the knife passing through the middle line of the embryo. Now, as for the grain of Corn, make out : (1) the **endosperm**, which occupies most of the section ; (2) the **embryo** with its broad **cotyledon** pressed against the endosperm, its pointed **plumule**, and its little **radicle**. Use a needle or a knife to help in distinguishing these parts.

Sketch the section just examined. Make the drawing several times the natural size, and name each part.

If you so desire, you may take home some seeds for further study or to complete any part of the work left unfinished.

CHAPTER III

THE MICROSCOPE

The following will be supplied :

- (1) A compound microscope ;
- (2) Lens paper, for cleaning lenses ;
- (3) A strip of paper 160 mm. long, for measuring the length of the tube of the microscope.

Illustrations.—Two illustrations of the microscope, one showing its mechanical features and another showing the path of the light rays, are provided in this book. You should make use of them in connection with the instructions which follow.

Definition.—A microscope is an optical instrument, containing a lens or a system of lenses, which is used for making an enlarged image of an object which is too minute to be viewed satisfactorily with the naked eye.

Do not try to use your microscope before you have read through the instructions, as it is an expensive instrument and you may damage it.

Microscopes are of two kinds : **simple** and **compound**.

THE SIMPLE MICROSCOPE

A simple microscope consists of a lens made of one piece of glass, e.g. a **pocket lens** or **magnifying glass**. The writer carries a pocket lens which magnifies four times (i.e. 1 mm. looks like 4 mm.). This lens is 1.25 inches in diameter, is biconvex, and its two surfaces are not strongly curved. There are other pocket lenses which are less in diameter, have more sharply rounded surfaces, and magnify more highly (up to 15 times) ; but they have to be put very near the object and have but a narrow field of view. With a simple lens, if made small enough and its surfaces rounded enough, one can obtain a magnification of 50 times ; but the image of the object is imperfect, owing to optical defects inherent in such a simple microscope. The optical defects are known as : (a) **chromatic aberration**, (b) **spherical aberration**, (c) distortion, and (d) curved field.

CHROMATIC ABERRATION

With light of mixed wave-length, e.g. diffused daylight (this contains light-waves of all the colours of the rainbow : red, orange, yellow, green, blue, indigo, violet), the shorter waves, e.g. blue, are brought to a focus in front of the longer waves, e.g. red (see a diagram provided or on the blackboard). Hence rings of colour tend to be formed about the image and the image is imperfect.

SPHERICAL ABERRATION

With monochromatic light, e.g. red light of a particular wave length, the rays refracted from near the edge of the lens come to a focus in front of those refracted from near the middle of the lens (see the diagram provided or on the blackboard). This, again, leads to the formation of an imperfect image.

THE COMPOUND MICROSCOPE

A compound microscope, e.g. the one provided, is so constructed that it gives a much higher magnification than can be obtained with a pocket lens and, at the same time, it is devoid of the optical defects of spherical and chromatic aberration.

Conrad Beck says : "The modern microscope is optically the most elaborate and the most interesting of all optical instruments." The lenses can be ground and fitted into each microscope only by highly skilled workmen, and the metal parts must be constructed so as to fit together with the greatest exactitude. Hence the microscope supplied to you has cost the University a considerable sum of money (about \$75.00). You must therefore **handle your microscope with the greatest care**, so as not to damage any of its parts ; and this applies particularly to the objectives.

PARTS OF THE COMPOUND MICROSCOPE

Make out the parts of the microscope one by one, beginning with the horse-shoe base and working upwards. Write down in your book a list of the parts as you make them out, and commit their names to memory. The parts are as follows :

1. **Horse-shoe base.** Some microscopes stand on a tripod (three legs), but the horse-shoe base is very compact and convenient. It has three **toes** and therefore rests firmly on the table.

Lift up the microscope and look under it at the toes. The horse-shoe form leaves a place for the mirror between the two arms of the shoe. If the base were just a flat sheet of metal, the mirror would have to be higher and then the tube would have to be higher, and the increased height of the instrument would be inconvenient. The horse-shoe base forms part of the **stand** of the microscope and by its weight increases the stand's rigidity.

2. **Pillar.** The pillar is a vertical rod fixed to the base and supporting the rest of the instrument.

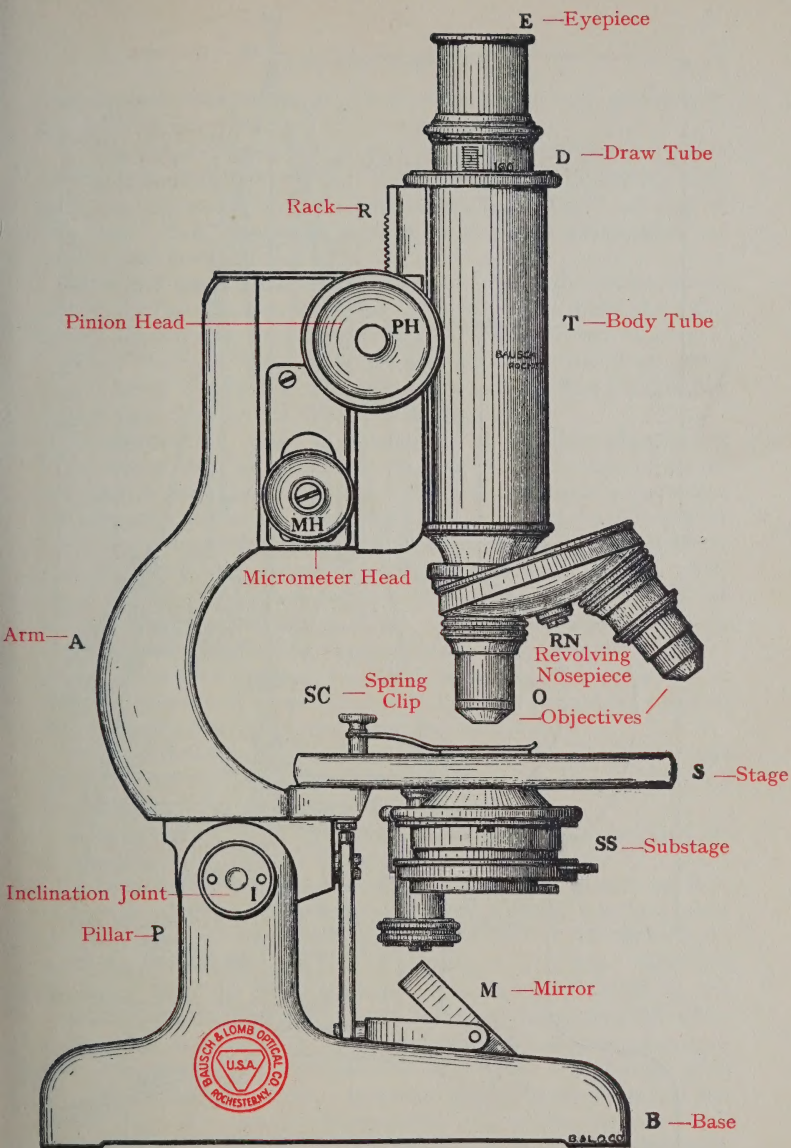
3. **Inclination joint.** This is at the top of the pillar and is used for tilting the microscope, when tilting is desirable. This joint is always made just stiff enough, so that, when the microscope is tilted at an angle, it will stay where it has been put. **Tilt your microscope** by holding down the base and pillar with one hand and pulling back the rest of the instrument with the other hand which should grasp the arm or handle-arm above the pillar.

When the microscope is tilted, the stage is tilted, and a slide placed upon it would slip off if not held down by the clips. For all the work to be done in this class the **tube of the microscope must be kept in a vertical position**. This position gives greater freedom in moving slides about on the stage with the fingers. **Restore the microscope** to its original position.

4. **Stage with its two Clips.** The stage is the square platform on which a slide is placed for the examination of some microscopic object. It is provided with two **clips** or removable metal springs which can be used to hold a slide down when the microscope is tilted or when a slide is to be shown at a *conversazione*, etc. For the work to be done this session, **the clips are not to be used**. When clips are applied to a slide, they prevent one from delicately moving the slide about with the fingers. Turn the clips back against the arm or handle-arm, so that they are away from the stage.

5. **Arm or handle-arm**, according to the type of your instrument. An arm is straight and a handle-arm is curved. Both of them are above the inclination joint and hold the tube of the microscope in its position. The handle-arm is of recent make and is convenient for carrying a microscope about.

6. **Micrometer head.** A brass screw-head at the top of the arm or handle-arm, used as a **fine adjustment** in raising and lowering the tube. In the newest microscopes there are two



Mechanical Features of Microscope

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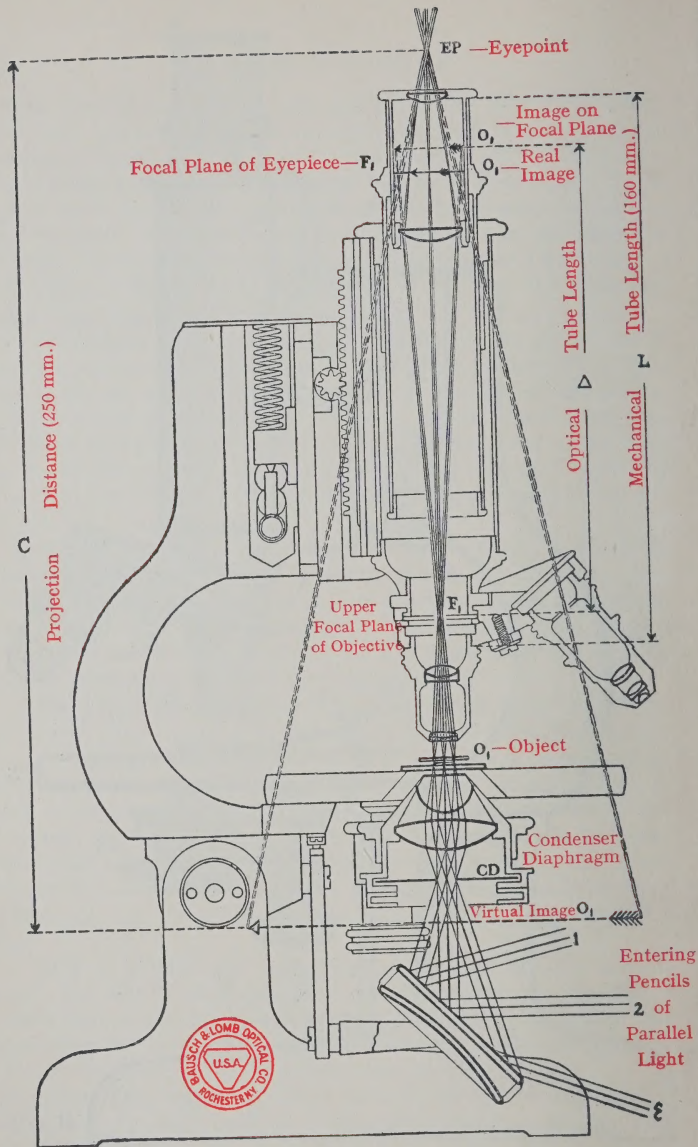


Diagram Showing Path of Light Rays

(Reproduced by permission of the Bausch & Lomb Optical Co.)

micrometer heads, one on each side of the handle-arm underneath the large pinion heads of the coarse adjustment.

7. Rack and Pinion. The rack is a toothed strip of metal attached to the body-tube. The pinion (a little wheel with teeth) is connected with the two **pinion heads** right and left of the rack. This apparatus is used as a **coarse adjustment** for raising and lowering the tube.

Raise and lower the tube by means of one of the pinion heads of the coarse adjustment. This is usually done with the right hand. Notice that the tube is caused to rise and sink rapidly.

When the tube is raised about an inch above the stage, raise it and lower it with the micrometer head of the **fine adjustment**. Notice that the tube rises and sinks very slowly.

To observe the range of movement of the fine adjustment. First, using the coarse adjustment, see that the tube is raised one inch above the stage (this to avoid accidentally screwing the objective down on to the stage). Then, using the micrometer head of the fine adjustment, screw upwards as far as the screw will go, and then downwards as far as the screw will go. The screw should then be set between these two extreme limits: it will then work either up or down. When the microscope is in use, the screw sometimes works gradually to its upper or lower limit. It is then necessary (after first raising the tube with the coarse adjustment) to set the screw in an intermediate position where, once more, it will work both up and down.

The coarse adjustment is used for getting an object into focus quickly with the low-power objective, and the fine adjustment for focussing with the high-power objective.

8. Body-tube. The outer tube (usually black, sometimes with a brazen surface) held by the arm or handle-arm.

9. Draw-tube. A tube inside the body-tube. About an inch below the top of the microscope tube you will find a milled collar (the first one you come down to). Take hold of this and gently **raise the draw-tube**, revolving it slightly in so doing. Find the **graduated scale** on the draw-tube. The standard length of the tube, i.e. the length which gives the best optical results when objects are examined, is 160 mm. On this account now push down the draw-tube until the 160 mm. mark is just level with the top of the body-tube. In most of the microscopes (all the newer ones) the distance between the top of the objective and the top of the eye-piece, i.e. the **tube-length**, will now be 160 mm.

In a few microscopes (the oldest ones) the tube will still be too long, as some years ago microscope makers did not allow 15 mm. for the addition of the nose-piece when this was first introduced. Therefore each one of you should **measure the tube-length for yourself**. For this purpose use your own measuring rod or, if you have not got one, the strip of paper provided. The strip is 160 mm. long (160 mm. = 6.3 inches).

A draw-tube **allows one to vary the length of the tube as a whole**. With high magnification (using the $\frac{1}{6}$ objective) the tube-length ought to be made **less than 160 mm.** when the object is covered by a very thick cover-glass (140 mm. with a cover-glass 0.2 mm. thick), and ought to be made **greater than 160 mm.** when the object is covered by a very thin cover-glass (240 mm. with a cover-glass only 0.1 mm. thick). In your work you will be given cover-glasses of intermediate thickness suitable for a tube-length of 160 mm. (No. 2 cover-glass, with a mean thickness of 0.18 mm.)

10. Ocular (eye-piece). Take hold of the collar at the very top of the draw-tube and **remove the ocular**. This is the **high-power ocular**. It can be pulled out very easily and, when put back, drops into the tube by its own weight, i.e. it is very loosely fitted. This enables oculars to be changed very quickly. **Never turn a microscope upside down**; for, if you do, the ocular will drop out and, should it fall to the ground, its usefulness will be destroyed.

Now that the ocular is out of the draw-tube **look down the open tube** and notice the **diaphragm** at its base. This is a metal disk with a large round hole in its centre. Its rim prevents light being reflected at the inner sides of the tube (see the illustration of the microscope supplied).

Examine the removed ocular. It is a metal holder containing two lenses with a diaphragm between them.

Carefully unscrew the upper lens of the ocular. This is the **eye-lens**. Keep it clean; do not put finger-marks upon it, as objects cannot be seen clearly through dirty lenses. Is the top surface of the lens flat, convex, or concave? Is the under surface of the lens flat, convex, or concave? Is the lens rightly described as plano-convex? The eye-lens can be used as a **magnifying glass**. Try it on print and then on your skin.

Look down the open eye-piece and notice the black **diaphragm**, i.e. a metal plate with a large round hole in it. It is situated (is it not?) slightly below the centre of the metal case. In other

oculars (see illustration of the microscope supplied) the diaphragm is much below the centre of the case. The **image** (picture) of the object is formed exactly **at the level of the diaphragm** (see the arrow in the illustration), and the metal rim serves to prevent unrequired light from passing up the tube. Later we shall make a simple experiment by means of which we shall be able to convince ourselves that there is an image of the object at the level of the diaphragm.

Clean the eye-lens by breathing on it and rubbing gently with some **lens paper** (special paper supplied, to be used only for cleaning lenses) or a clean handkerchief. A gentle rub will not scratch the lens and will remove any finger-marks. Note that the surfaces are clean by looking at them with light reflected from the window. Never rub a lens more than is just necessary to clean it.

Carefully, without any forcing (which might ruin the thread), **screw back the eye-lens into the metal case**. It is best to do this by screwing backwards a trifle (counter-clockwise) to set the thread, and then screwing forwards (clockwise). Let the lens **just go home**. Do not tighten it beyond that.

Now remove the lower lens of the ocular. This is the **field-lens** (or **collecting lens**). As before, make out the shape of its two surfaces. Is the lens plano-convex like the eye-lens, but of greater diameter? Can it be used as a magnifying glass? **Clean** the field-lens and screw it home (no forcing).

Make a drawing of a vertical section through the eye-piece (see the illustration in this book or on the blackboard). Indicate the **metal holder**, the **eye-lens**, the **field-lens**, and the **diaphragm**. A ruler should be used to draw the flat surface of any lens.

Each microscope has two interchangeable oculars :

- (a) **Low-power ocular**, No. 5 (in some instruments No. 2).
- (b) **High-power ocular**, No. 10 (in some instruments No. 1).

The low-power ocular is longer than the high-power (compare their lengths), has a larger eye-lens, and gives a **smaller magnification**. Hence the nomenclature.

11. Objective. An objective is a system of lenses held in a brass case and placed a little way above the **object** to be examined. Your microscope is provided with two objectives attached to a nose-piece at the base of the body-tube. These are :

- (a) The **low-power objective**, marked 16 mm. ($\frac{2}{3}$ inch) ;
- (b) The **high-power objective**, marked 4 mm. ($\frac{1}{6}$ inch).

Find the marks on the two objectives. (*a*) has a wider lens at its base and gives a **lower** magnification than (*b*). Hence the nomenclature of the two objectives.

With all due care, **unscrew the two objectives** and so remove them from the nose-piece (do not drop them on the table). To do this, first turn the microscope sideways; then hold the nose-piece with your left hand and unscrew each objective in turn with your right hand. There is a **double-milled collar** at the top of each ocular close under the nose-piece. Put your finger and thumb on this to do the unscrewing (turn in the counter-clockwise direction). Do not use the single-milled collar in the middle of the high-power objective or you may only remove the lower half of the objective and this is very undesirable. The oculars are **not to be taken to pieces**, as they are very delicately adjusted and you may not be able to put them together again with the exactitude attained by the maker. Also, if they are not opened, dust cannot get into them.

In your **low-power objective** there are **two lenses** (see the diagram in this book or on the blackboard). Each lens is plano-convex (flat below and convex above) and is made up of **two pieces of glass**, the lower piece being plano-concave and the upper piece biconvex. The two pieces of glass in each lens are made of different materials which have **different optical properties**. The combination of two such compound lenses in the objective **corrects the optical defects** always present in a single simple lens and already referred to as chromatic and spherical aberration. Both your objectives are **achromatic**.

In your **high-power objective** there are **three lenses**. The lowest one is very narrow and is composed of one piece of glass only, while the middle and upper lenses are each made up of two pieces of glass (see the diagram in this book or on the blackboard).

In very high-power objectives, e.g. $\frac{1}{18}$ inch (used for bacteriology, etc.) there may be as many as five lenses made out of ten pieces of glass (the uppermost lens containing three pieces). Such an objective can only be made by very skilled mechanics, and may cost more than the **stand** of the microscope together with all its other **attachments** (oculars, objectives, sub-stage with its condenser, etc.).

Note that the low-power objective can be used as a magnifying glass. Try it on print. It must be held near the paper, if you are to see anything.

Make a **drawing of a vertical section through the low-power objective** (see the diagram in this book or on the blackboard). Indicate the **metal holder** and the two plano-convex compound **lenses** (upper and lower).

Clean the lower lens of each objective by breathing on it and rubbing lightly with lens paper or a clean handkerchief, and see that the surface rubbed is clean by looking at it with light reflected from a window.

Now **screw the objectives into the nose-piece**. Put the **low-power** one back first. It has 16 mm. ($\frac{2}{3}$ inch) marked on it and must be **put back into the hole marked 16 mm.**, and not into the other hole. Be careful to screw each objective in gently and only just home (no forcing or you may spoil the thread of the screw). Do not hurry at first, it takes a little time to fit each objective into its thread. If in difficulty, ask the help of a demonstrator.

Note that the marks **16 mm.** and **4 mm.** on the low-power and high-power objectives are the **focal lengths** of the objectives. By **focal length** is meant the distance of the object examined from the optical centre of a lens or system of lenses when the object is in clear focus. This distance is 16 mm. for the low-power objective but only 4 mm. for the high-power objective. In each objective the optical centre is at some distance above the bottom of the lowest lens.

The **working distance** for an objective is the distance between the lowest lens of an objective and the object examined, when the object is in focus. Note that the focal length and the working distance of an objective are not identical. Thus the low-power or 16 mm. ($\frac{2}{3}$ inch) objective has a focal length of 16 mm. ($\frac{2}{3}$ inch), but its working distance is only 5 mm. ($\frac{1}{5}$ inch); while the high-power or 4 mm. ($\frac{1}{6}$ inch) objective has a focal length of 4 mm. ($\frac{1}{6}$ inch), but its working distance is only 0.3 mm. ($\frac{1}{80}$ inch). In finding an object we always use the low-power objective first, in part because it has such a large working distance.

12. Revolving nose-piece. This is the metallic attachment (round in the newer instruments) which projects at the base of the body-tube and holds the two objectives. When an object has been brought into focus with the low-power objective, one can revolve the nose-piece and thus change the low-power objective for the high-power. **Revolve the nose-piece.** Note that when an objective gets into its right place under the body-tube there is a little **click** announcing the fact. The black

stationary **disk** which covers the top of the nose-piece (in most instruments) prevents dust from falling into the objectives when they are being rotated. The nose-piece is a modern invention, the use of which saves much time in changing from the low-power objective to the high-power. Some nose-pieces instead of being double are triple, i.e. hold three objectives instead of two.

13. **Mirror.** The mirror is beneath the condenser under the stage. Note that it is **plane** on one side and **concave** on the other, and that it can be moved from side to side and also be rotated. These two movements allow one to adjust the mirror in any way that is desirable. The mirror is **used for reflecting light** coming from a window on to the object to be examined and up the tube to the eye.

14. **Sub-stage.** A metal structure beneath the stage which holds :

(a) An **Abbé condenser** made up of two large lenses (see the diagram in this book or on the blackboard), used for **concentrating light** coming from the mirror on to the object to be examined.

(b) A **lower iris diaphragm**. This has a little bar-handle, so that it can be opened or closed, thus admitting more or less light to the object and up the tube. Later on you will make much use of this diaphragm.

(c) In most of your instruments there is also an **upper iris diaphragm**. This can only be used when the condenser has been removed from the sub-stage, and it is never employed in the work to be done in this class. For our work it is a superfluous attachment. **Do not attempt to use it** when the condenser is under it, as it is impossible to shut it under this condition and an attempt to shut it is likely to injure it.

Examination of the sub-stage and its attachments. The sub-stage is attached to the stage by a metallic rod on the left side of the under part of the stage. Beneath this rod there is a screw with a milled head. Using this screw, **screw down the sub-stage** as far as it will go, and then **swing the sub-stage out** for examination. Now **tilt the microscope** through nearly a right-angle and **remove the condenser and iris diaphragm** from the metal holder. Ask a demonstrator to show you how this is done. Both hands are required for this operation.

After the condenser and iris diaphragm have been removed,

carefully examine both these structures. Open and close the iris diaphragm gently and note that the aperture in its centre can be expanded or contracted with great delicacy, thus allowing more or less light to pass through it (compare the **iris** in the eye). Note the size and position of the **handle** of the iris diaphragm, as you will use it for adjusting the amount of light in all your future work. The upper iris diaphragm (where present) has been left in the metal holder and can be worked in the same way as the lower one, but it must be **fully open** when the condenser is put back into position or it will be in the way and be injured. Note that under the lower iris diaphragm there is a **slot**. Into this one can put a sheet of blue glass to cut off any undesirable yellow rays in which artificial light is usually rich.

At the side of the metal holder of the sub-stage notice a screw which, if necessary, can be used to press the metal holder against the condenser and thus prevent the condenser from falling.

Clean the upper and lower lenses of the condenser in the usual way (leave no finger-marks on them). Then **fully open** the iris diaphragm still on the microscope at the top of the metal holder. Now **put back the condenser** with its iris diaphragm into the metal holder, the **handle** of the iris diaphragm being directed toward you. To get the condenser to go back quite home it is necessary to press the handle of the upper iris diaphragm back (so as to open the diaphragm as widely as possible) during the operation. If in difficulty about this matter, ask the help of a demonstrator. Finally, **swing the sub-stage back** beneath the stage and **screw it up** until the upper surface of the top lens is on a level with the stage.

Make a drawing of a vertical section through the condenser (see the diagram in this book or on the blackboard). Indicate the **metal holder** and the **upper and lower lenses**.

How many pieces of glass are there in your microscope, including the two oculars and both objectives? Write down the answer in your book and indicate where each piece of glass is located.

CHAPTER IV

HOW TO WORK WITH THE COMPOUND MICROSCOPE

For your work to-day you will be provided with :

- (1) A compound microscope ;
- (2) A glass of water ;
- (3) A glass rod ;
- (4) A needle mounted in a wooden handle (do not break it) ;
- (5) Several microscope slides ;
- (6) Some cover-glasses (do not put finger-marks on them) ;
- (7) Cheese-cloth or some other fabric for wiping slides and cover-glasses when necessary ;
- (8) Lens paper, to be used only for cleaning lenses ;
- (9) A piece of blotting-paper, for soaking up any excess water around the cover-glass when a preparation has been made ;
- (10) A piece of a Potato tuber ;
- (11) A small triangular piece of black paper ;
- (12) A small round disk of translucent lens paper ;
- (13) A strip of paper 160 mm. long or a measuring rod.

Use of the Microscope. It is important to learn once and for all how to use the microscope properly. Therefore, carefully follow the instructions to be given. You are now : (1) to make a preparation of starch grains, (2) to set the microscope in the right position, (3) to obtain proper illumination, and (4) to bring the object (starch grains) into focus under the low-power objective.

1. TO MAKE A PREPARATION OF POTATO STARCH GRAINS

Take one of the plain glass microscope slides provided, and place it on the table before you.

Take the piece of Potato provided, slice it across so as to expose a **fresh moist surface**. Then from the surface, with the help of the mounted needle or a knife-blade, lightly scrape off a **very little** of the turbid **sap** on to the middle of the slide (the drop of sap should not be more than $\frac{1}{4}$ inch wide). This sap contains starch grains which were stored in the cells of the Potato tuber.

With the help of the glass rod provided, add a small drop of

water to the sap on the slide. The drop, after deposition, should be about $\frac{1}{8}$ inch or 1 cm. wide.

Using the needle, stir up the drop so that the starch grains may become distributed in it.

Take up one of the cover-glasses, and, holding it by its edge, look at it by reflected light to see if it is clean. If it has dirt or finger-marks upon it, hold it by its edge between a finger and thumb of the left hand, and put it between the lips of your open mouth, and breathe on both sides of it. Then with a finger and thumb of the right hand rub both of its surfaces at one and the same time, using the wiper provided or a clean handkerchief which is very good for this purpose. Do **not** use lens paper. If you rub lightly in the manner described, the cover-glass will not break. After the cover-glass has been rubbed, look at it again by reflected light to make sure that it is now clean. **Before using a cover-glass, always see that it is clean**, as a dirty cover-glass prevents one from seeing an object clearly.

Now put the cover-glass on the top of the drop. To do this without including large air-bubbles in your preparation, hold the cover-glass **obliquely** just over the drop so that one side is lower than the other; then lower the cover-glass until the lowest side touches the edge of the drop; and then let the whole cover-glass fall on to the rest of the drop. Note with the naked eye whether or not larger or smaller air-bubbles have been accidentally included in the preparation. The more free the preparation from these bubbles, the better has it been made.

If your cover-glass is **floating** on the drop, too much water was originally placed on the slide. Excess water must then be removed with blotting-paper. Enough water should be left **to fill the space under the cover-glass**. If the water does **not** completely fill the space under the cover-glass you must **add some more**. To do this, put another small drop of water on the slide **near** the cover-glass **but not touching it**. Then, with your needle, join the drop to the edge of the cover-glass. It will then be sucked under by capillary attraction without any of it going on to the top of the cover-glass.

It is possible to get rid of air-bubbles from a preparation by lifting up the cover-glass on one side with a needle and by then letting it fall again, provided sufficient water is present.

It is important to **keep the top of the cover-glass** in any preparation **clean and free from water**. If a cover-glass should get wet

above, you must put the preparation on the table, remove the cover-glass, dry both sides of it, and then replace the cover-glass on the drop, adding more water if necessary. If the top of a cover-glass gets wet, the water is likely to wet the lower lens of the high-power objective when in use, and this is highly undesirable.

A preparation made with water slowly **dries up**, and air passes beneath the cover-glass. **Look at your preparation from time to time** and, as necessary, add more water to it. If it dries up too much, the cover-glass will be dragged down toward the slide and the starch grains may thereby become crushed and broken. The space under the cover-glass should be kept full of water. If the starch grains should become crushed, you must make a fresh preparation.

2. TO SET THE MICROSCOPE IN THE RIGHT POSITION

See that the tube-length of your microscope is 160 mm., and that the low-power objective is about $\frac{1}{4}$ inch from the stage. Set the microscope so that the point of the horse-shoe base is directed toward you and is **at the very edge of the table**, and so that the two arms of the horse-shoe are pointed directly away from you. The microscope stand is not to be directed toward a window (unless a window happens to be straight in front of you). Now set your stool close up to the table. Then sit upright upon it. Even if you are short in stature, you will be able to see down the microscope without difficulty.

3. TO OBTAIN PROPER ILLUMINATION

This is a very important detail and should always be carefully done. Theoretically, when a microscope is provided with a condenser, the **plane** (flat) mirror should be employed (see the diagram in this book). Therefore, **turn your plane mirror uppermost, in preparation for use.**

Place both arms on the table and grasp the mirror with the fingers and thumbs of both hands. Now look down the microscope tube. The right eye is usually employed for this purpose. The other eye is always kept **open** by all experienced microscopists. **Learn not to close the other eye.** Now move the mirror about, using both hands, and turn it so that it reflects

light from the nearest window up the tube to your eye. The way in which rays of light are reflected from a plane mirror is shown in the diagram.

When the mirror has been properly adjusted, the **field of view**, as seen through the tube, should be **uniformly lighted with white light**. But, as you are some distance from the window and the panes are small, you may see dark **window-bars** in your field. **Remove the ocular** for a moment and look down the tube through the objective. You will then easily see whether or not you have **central** illumination and **even** illumination over the whole field. If necessary, adjust the mirror before replacing the ocular.

The **window-bar images** seen in the field of view can be got rid of by using the **concave** mirror instead of the plane mirror. Try substituting the concave mirror for the plane mirror. While, with a suitable source of light, e.g. day-light coming through a large window-pane when the microscope is close to it, the plane mirror gives the best optical results, particularly with high-power objectives; yet, under such conditions as those with which you are now working, if the window-bar images should interfere too much with a clear vision of the object, it may seem better to use the concave mirror instead of the plane. You may use your own judgment in this matter.

The **amount of light** coming up the tube is of great importance and, whenever an object is examined, **must be regulated** by means of the handle of the iris diaphragm on the right-hand side of the base of the sub-stage. The handle can be moved through a quarter circle. Use it with your right hand whilst you look down the microscope. Open the diaphragm to its fullest extent, and then close it as far as it will go without forcing. Finally, open the diaphragm slightly and leave it thus.

4. TO BRING THE OBJECT INTO FOCUS WITH THE LOW-POWER OBJECTIVE

Place your preparation of Potato starch grains on the stage of the microscope, so that the part you wish to examine is at or near the centre of the stage above the centre of the condenser. Since the stage is horizontal, the slide rests there without slipping. The **clips** are **not to be used**, as they would hinder your freedom in moving the slide about with the fingers. The clips should be turned backwards out of the way.

To focus an objective on an object is to adjust its relation to the object so that a clear image is obtained. Focussing should involve no danger to the front lens of the objective or to the cover-glass by their coming into violent contact. To focus the starch grains in your preparation proceed as follows :

Lower the head to the level of the stage (on the left side) and look horizontally at the lowest part of the objective. Then with one hand (the right) lower the tube with the coarse adjustment until the objective is within $\frac{1}{4}$ inch of the cover-glass. Then look down the tube and **slowly elevate the tube** by means of the coarse adjustment until the image of the starch grains becomes distinct. Then, to improve the focus, use the micrometer head of the fine adjustment. By focussing **upwards** in the manner just described there is no risk of injuring the objective or breaking the cover-glass. In your future work, when you examine an object, always find focus with the low-power objective by first lowering the tube and then raising it in the manner described.

Adjusting the amount of light. The starch grains may be too strongly illuminated so that their details are blurred, or too weakly illuminated so that they are not clearly seen. **Regulate the light with the iris diaphragm** so as to meet the requirements of your own eye. Note how much sharper the outlines of the starch grains become when the light is cut down.

Practice finding focus with the low-power objective. Do this by first raising the tube, say two inches above the objective, and by then following the directions given in the second paragraph on this page. Obtain focus three times in succession. Practice increases the rapidity with which this can be done.

To find the best part of the preparation. With your arms resting on the table, take hold of the two ends of the slide with a finger and thumb of each hand and, looking down the microscope, move the slide about on the stage and find the best place in the preparation. **Look all over the preparation.** In one place, perhaps, the starch grains are touching one another and therefore are **too crowded**. In another place, perhaps, the starch grains are **too far apart**. The best part of the preparation is where there are plenty of starch grains sufficiently separated from one another to be seen individually.

You may have some small **air-bubbles** in your preparation. These are rounded, as a rule much larger than starch grains, and their outlines alter in a characteristic way when the tube is

raised and lowered. Note that, when you move the preparation to the right, it seems to move to the left, and *vice versa*.

A **Magnification Table** will be given later, but it may now be noted that, when the starch grains are in focus with the high-power ocular (No. 10, the one in use) and the low-power objective (16 mm.), they are magnified 100 diameters.

Make a **sketch** showing a number of starch grains. The largest should be drawn about $\frac{1}{3}$ inch long. Represent small grains as well as large ones. Aim at accuracy in outline. Do not draw a circle to indicate the edge of the field of view. Note the relative sizes and shapes of the grains. Write above your drawing: **Potato starch grains seen with the low-power objective.**

5. TO BRING THE OBJECT INTO FOCUS WITH THE HIGH-POWER OBJECTIVE WITHOUT USING THE NOSE-PIECE

Using the low-power objective, first see that there are some starch grains in sufficient number in the middle of the field of view.

Raise the tube two inches above the slide with the coarse adjustment. Revolve the nose-piece so as to put the **high-power** objective at the end of the tube.

Now **lower the head to the level of the stage and look horizontally** between the objective and the cover-glass. Carefully lower the objective with the coarse adjustment until its front lens is **nearly in contact** with the cover-glass, **but not quite**. Then look down the ocular, and **very slowly** elevate the tube by the coarse adjustment until the image of the starch grains appears. Then, to get perfect focus, use the fine adjustment. Note how very close to the cover-glass the high-power objective is when the starch grains are in focus.

It may be that in your preparation, as seen with the high-power objective, there are no starch grains sufficiently in the centre of the field of view. If so, move your preparation about with the two hands in the manner already described until some grains have been brought into a central position. With practice it is possible to do this with great delicacy.

Adjust the amount of light required to see the starch grains clearly. The diaphragm will doubtless need to be opened more widely than for the low-power objective, so as to admit more light.

For practice, repeat once or twice the process of focussing the starch grains with the high-power objective.

The magnification of the starch grains with the high-power ocular (No. 10) and the high-power objective (4 mm.) is 430 diameters.

6. A STUDY OF THE STRUCTURE OF A FEW STARCH GRAINS

Note the difference in shape between the large and small grains. The organic centre of a grain is called the **hilum**. Around this the grain has been built up by protoplasm from sugar layer by layer, so that the larger grains are clearly **laminated** (made up of **laminae**). When small, every grain is round in outline; but, as a grain grows, each new layer (lamina) built up on its exterior becomes more and more eccentric on account of the fact that it is thicker on one side than on the opposite side. Note that, to observe the laminae well, **the light must be carefully adjusted by means of the iris diaphragm**. The largest grains show lamination best, as one might expect, as they consist of the most layers. Potato starch grains are distinguished from those of the Pea, Wheat, and other plants by their peculiar shape and size.

Make a **sketch** of a few starch grains on a larger scale than before, so as to show details of structure. The largest grains should be drawn fully 1.5 inches long. Indicate the **hilum** and the **laminae**. You may happen to observe some **compound grains**, but there is not time to study them.

7. THE NOSE-PIECE AS A MEANS OF CHANGING THE OBJECTIVES

With the help of the nose-piece, turn on the **low-power** objective and carefully focus some starch grains in the middle of the field of view. Then, by revolving the nose-piece, **slowly and carefully change the low-power objective for the high-power**. With most of your instruments this can be done without the high-power objective touching the cover-glass. If, however, as you revolve the nose-piece, the high-power objective, **owing to an imperfection in your instrument**, should come into contact with the cover-glass and thus be prevented from taking its proper place at the base of the tube, you must **raise the tube** a little with the coarse adjustment so as to allow the objective to go home.

After the high-power objective has been swung into position the starch grains may be more or less in focus. If they are, bring them into perfect focus by means of the fine adjustment. **If the starch grains cannot be seen even as blurs, raise the tube gradually with the fine adjustment. Turn the micrometer head in the counter-clockwise direction.** If you screw in a clockwise direction, you will lower the tube and force the objective against the preparation with disastrous results to your preparation and the objective. There is never any danger to your instrument if you **focus upwards.**

If, after raising the tube about $\frac{1}{10}$ inch, **the starch grains do not come into view,** lower your head to the level of the stage, then lower the objective until it is nearly in contact with the cover-glass, but not quite, then look down the tube and focus slowly up again. The starch grains will then come into view.

In some of your microscopes, perhaps, when after focussing starch grains carefully with the low-power objective you switch on the high, the tube needs **lowering** instead of raising to bring the starch grains into focus. However, **it is always risky to screw the high-power objective down** as it is so near the cover-glass. When an experienced microscopist has to do this, he **moves the slide about slightly with the left hand** while he lowers the tube very slowly and cautiously with the right hand. Moving the slide slightly enables one to perceive more easily when the object is coming into view; also, should the tube be screwed down too far so that it comes into contact with the cover-glass, the contact is at once felt with the left hand and then one at once raises the tube again.

Each microscope has its own individuality, and only trial will show whether, after switching on the high-power objective, the tube requires to be slightly raised or slightly lowered to bring the object into focus. In any case, **be careful to avoid screwing down the tube so far that the objective presses on the cover-glass.** Should this pressure take place, the starch grains will be crushed and your preparation thus spoiled, and it will be necessary for you to make a fresh preparation.

With a little practice, using the nose-piece, one can pass very quickly from focus with the low-power objective to focus with the high-power, and *vice versa*. From now onwards you should use the nose-piece as a time-saver.

8. PARFOCALITY

In a perfect instrument, when the tube-length is 160 mm. and an object is in focus with the low-power, on switching on the high-power with the help of the nose-piece the object is again in focus. The two objectives are then said to be **parfocal**.

To make the two objectives parfocal. If with your tube-length 160 mm. the objectives are not parfocal, it is possible by using the draw-tube to make them so. The method is as follows :

With the **high-power** objective and adjusting the light as required, carefully **focus a particular starch grain** or a small group of grains in the middle of the field. Now switch on the **low-power** objective. Look down the tube. Are the starch grains you focussed now in focus ? If they are or are almost so, the two objectives are already sufficiently parfocal. If the starch grains are not in focus, then, without touching the coarse or fine adjustments, **raise or lower the draw-tube** as may be required until the starch grains are in perfect focus. When this has been done, **the two objectives are parfocal**, and one can change from one to the other and the object will always be in focus.

If your instruments were all perfect, the objects would be parfocal with a tube length of 160 mm. ; but some of them may be imperfect, usually owing to a defect in the fitting of the nose-piece, and in these, if one makes the objectives parfocal, one has to sacrifice the best tube-length, which is 160 mm. It is therefore **best to keep the tube-length at 160 mm.** and to learn how to manage your microscope so as to find focus with the high-power when this has been exchanged for the low-power. We shall shortly prove by experiment that, for the normal magnifications for which the instrument has been designed, a tube-length of 160 mm. does, as a matter of fact, give the clearest image of the object.

9. THEORY OF THE MICROSCOPE

The compound microscope is an optical instrument by means of which a system of lenses in the objective produces a magnified image of an object, which image is viewed through the eye-lens of the ocular that still further enlarges it.

An objective is an **image producer** (see the diagram of the microscope). The eye-lens of the ocular is an **image-viewer** and an **image-magnifier**. The field-lens of the ocular is a **collecting**

lens : it collects the rays of light coming from the objective and brings them to a focus so that they form **an image of the object exactly at the level of the diaphragm** of the ocular when the tube-length is 160 mm. This image at the level of the diaphragm is exactly in the right focal plane for examination through the eye-lens.

To prove by experiment that the objective and the field-lens of the ocular produce an image of the object at the level of the diaphragm of the ocular. Take a microscope slide. Put it on the table before you and place upon it a triangular piece of **black paper** (provided). The paper should be quite flat.

See that the tube-length is 160 mm. With the low-power objective focus the sharpest angle of the piece of paper on the slide. The point of the angle should be at about the middle of the field of view.

Now remove the ocular and take off its eye-lens. Let a flat disk of translucent paper, cut the right size, fall down the open ocular tube so that it comes to **rest on the diaphragm**. Lens paper or tracing paper is suitable for this purpose (a piece of one of them has been provided).

Now **lower the open ocular down the tube** to its normal position. You must hold it with a finger to prevent it from falling to the bottom of the tube (a mounted needle or knife pressed against the screw-thread just inside the ocular may be used instead of a finger to prevent falling).

Now, with your eye about 10 inches above the ocular, look down the tube. You will perceive a **clear image of the piece of black paper** on the translucent disk of paper **at the level of the diaphragm**.

Now raise the ocular in the tube. The more it is raised, the less distinct is the image at the diaphragm. Similarly, if you lower the ocular in the tube beyond its normal position, the lower the ocular the less distinct is the image at the diaphragm. Evidently the **clearest image is obtained with a tube-length of 160 mm**.

Now remove the open ocular and, **leaving the disk of translucent paper on the diaphragm**, replace the eye-lens. Replace the ocular in the tube of the microscope. Look down the tube. You will now see not only the image of the piece of black paper on the paper disk at the diaphragm, but you will also be able to **see the structure of the paper disk**. This shows that the image

at the level of the diaphragm is **just in the right position** (the right focal plane) to be viewed and magnified by the eye-lens.

10. MAGNIFICATION

Magnification Table

OBJECTIVES	OCULARS	
	No. 5	No. 10
16 mm. ($\frac{2}{3}$ inch)	50	100
4 mm. ($\frac{1}{6}$ inch)	215	430

Each microscope is provided with a low-power ocular (No. 5) and a high-power ocular (No. 10) as well as with a low-power objective (16 mm.) and a high-power objective (4 mm.). Hence it is possible for you to magnify an object 50, 100, 215, or 430 times.

Magnification is always measured in diameters, and never in areas. A starch grain observed with ocular No. 10 and the 4 mm. objective appears to be 430 times as wide as it really is.

Change the oculars and note the change in the magnification of some starch grains.

For your **future work** you will be provided only with the **high-power** ocular (No. 10). Hence you will be able to use two magnifications only, namely, 100 and 430.

11. NUMERICAL APERTURE

On the 16 mm. objective you will find the figures 0.25 and on the 4 mm. objective the figures 0.65. These numbers indicate **numerical aperture**.

Resolving power is the property by which an objective shows distinctly separated two small elements in the structure of an object, which are only a short distance apart. **The measure for the resolving power is the numerical aperture (N.A.).** The higher the N.A., the greater the resolving power of the objective and the finer the detail it can reveal.

CHAPTER V

POTATO STARCH GRAINS AND THEIR MICROCHEMICAL REACTIONS. THE PULP-CELLS OF AN UNRIPE BANANA FRUIT

For your work to-day, in addition to a microscope, slides, cover-glasses, etc., you will be supplied with the following material and reagents :

- (1) Potato starch, in the form of a white powder ;
- (2) A green, unripe Banana fruit ;
- (3) Iodine, which colours starch blue and proteins yellow ;
- (4) Caustic potash (KOH), 10 per cent. solution ;
- (5) A drop of strong sulphuric acid (H_2SO_4) given out by a demonstrator ;
- (6) An alcohol lamp ;
- (7) A test-tube containing Potato starch paste.

A. FOUR RULES FOR THE USE OF THE MICROSCOPE

1. **Successive Use of the Objectives.**—Each object to be studied should always **first** be observed with the **low-power** objective, and then, if desired, with the high-power objective. With the low-power objective a good place in the preparation should be found and set in the centre of the field of view, so that when the high-power is turned on there will be something worth studying in the field of view.

2. **Removal of a Slide from the Stage.**—If a slide is on the stage of the microscope under the **high-power** objective, **before removing the slide always turn on the low-power objective.** This will provide plenty of space for the removal and prevent any liquid from the edge of the cover-glass getting on to the high-power objective. The slide should be **slid off the stage, not lifted off.** This can readily be done by pressing down on the slide with a thumb and drawing the slide from under the objective. Try this.

3. **Necessity of Keeping Lenses Clean.**—You should **examine your lenses from time to time** to see whether or not they are clean. If the ocular has finger-marks upon it, clean it by breathing on it and rubbing with lens paper. To examine the objectives, these must be **removed from the nose-piece** and looked at by reflected light from a window. With a little practice

this can be done quickly and easily. With the left hand the nose-piece is held firmly, and with the right hand an objective is unscrewed by means of its upper **double collar**.

4. How to Leave the Microscope at the Conclusion of the Day's Work.—Turn on the **low-power** objective. **Remove the slide from the stage.** By doing this the microscope will be ready for examining an object with the low-power objective the next time it is used; and, when the microscope is put away, there will be no slide to fall off the stage and be broken.

B. MICROCHEMICAL REACTIONS WITH STARCH GRAINS

The science of Chemistry may be divided into **Macrochemistry**, which includes all chemical operations performed in laboratories and factories on a relatively large scale with the naked eye, and **Microchemistry**, which includes all chemical operations carried out on a very small scale with the help of the microscope. Microchemical reactions are exceedingly valuable in the study of the cell-walls and cell-contents of plants and animals, and are in frequent use for determining the exact constitution of many food substances, drugs, fibres, etc.

To make a Preparation of Potato Starch Grains.—Take a microscope slide, place it on the table before you and, using a glass rod, deposit a small drop of water (about $\frac{1}{8}$ inch or 1 cm. wide) on the middle of the slide.

Wet the end of a mounted needle in water, and then **touch the wetted part to the Potato starch provided.** An amount of the white powder sufficient for your purpose will cling to the needle. More might be too much and result in the starch grains in your preparation lying too close together. Now **stir the starch** on the needle **into the drop of water** on the slide, and add a cover-glass in the manner already described in Chapter IV. See that the space under the cover-glass is just filled with water. Add more water if necessary, and remove any excess water around the edge of the cover-glass with the help of blotting-paper.

Examine the preparation with the **low-power** objective. **Move it about well** to see what it is like and whether or not it is satisfactory. Remember to **control the amount of light** with the iris diaphragm. You should perceive a clear image of the starch grains, and the grains should be sufficiently numerous but, for the most part, not in contact with one another. If your

preparation is unsatisfactory, make another. You should now be able to do this in a few seconds.

Reaction No. 1. Add Iodine.—The iodine supplied is dissolved in a solution of potassium iodide (KI). Iodine is only slightly soluble in water, but is much more soluble in potassium iodide. Hence the use of a solution of this salt as a solvent for iodine.

Place your preparation on the table. With the pipette attached to the stopper of the iodine bottle, deposit a small drop of iodine **near** the cover-glass, but not touching it; and then join the iodine to the edge of the cover-glass with a needle. The iodine will pass under the cover-glass by capillary attraction and diffusion, and none of it will get on to the top of the cover-glass.

With the naked eye, look at your preparation against a white background, and observe how far the iodine has penetrated under the cover-glass. Do the starch grains which have been acted on by the iodine appear as black specks?

If one desires to draw the iodine farther under the cover-glass, this can readily be done by applying blotting-paper at the edge of the cover-glass on the side opposite to the iodine. The **iodine should not be drawn more than half-way across the space under the cover-glass.**

Now put the slide on the stage of the microscope, and move it about with the fingers and thumbs of both hands until you **find the zone between the stained and unstained portions of the preparation.** Study this zone carefully. Observe how the iodine diffuses farther and farther into the field of view and alters the colour of each starch grain it comes to. Each starch grain absorbs iodine strongly. Does a starch grain first turn blue or violet and finally blue-black? **Write down the nature of the reaction** in your book.

If you so desire, observe some of the starch grains which are changing colour with the **high-power** objective.

Reaction No. 2. Add 10 per cent. Caustic Potash (KOH).—Turn on the low-power objective. Remove the slide from the stage. This slide will now be discarded.

Make a fresh preparation of starch grains. Put a drop of water on a slide and add starch as before, and then, **before** putting on the cover-glass, **add a drop of iodine** to colour the starch grains. Stir with a needle, and then put on the cover-glass. All the starch grains will now be blue. We have coloured them simply for the purpose of making them more readily seen after the next operation.

All microchemical work must be done on the table and not on the stage of the microscope, lest injurious chemical substances should get on to the stage or objectives and injure them.

Now, with the slide on the table, deposit a drop of 10 per cent. caustic potash (a strong alkali) on the slide near the cover-glass but not touching it, and then join it up to the edge of the cover-glass, so that it will be sucked under and not wet the upper surface. If the caustic potash should get on to the cover-glass, you must make a fresh preparation.

Look with the naked eye at the slide and observe how far the caustic potash has travelled. Then, with the low-power of the microscope, **look for the junction zone** between the caustic potash and the rest of the preparation, and observe what is going on. Control the amount of light, as required. Do the starch grains under the action of the alkali swell up or diminish in size? Does their colour increase or fade? At first, does the lamination of each grain affected become more or less distinct? Do the starch grains gradually lose their sharp outlines and melt into one another? **Write** down the nature of the whole reaction of the starch grains to caustic potash in your book.

Caustic soda (NaOH), another strong alkali, acts on starch grains in the same manner as caustic potash (KOH).

Discard the slide. Put it in the saucer.

Reaction No. 3. Add Strong Sulphuric Acid (H_2SO_4).—Make a **fresh preparation** of Potato starch grains with a drop of water and a cover-glass. **No iodine** is to be added. Remove any excess water around the edge of the cover-glass with blotting-paper.

Strong sulphuric acid is a very powerful reagent. If it gets on to clothes, it makes holes in them; if on to the skin, it causes a severe burning sensation; and if on to a microscope, it injures it. To avoid accidents, the **acid will be brought to you** by a demonstrator. When your slide is ready for the acid, put it near your table number on some white paper, so that the demonstrator may see the edge of the cover-glass readily. A small drop of acid will be set in contact with the cover-glass with the help of a glass rod. The acid would corrode a needle.

The sulphuric acid will diffuse under the cover-glass just as did the iodine and caustic potash. With the naked eye observe where the sulphuric acid has advanced to in your preparation. Then put the slide on the stage and **find the junction zone** with the low-power objective. Adjust the light carefully.

How does the sulphuric acid affect the starch grains? Make this out by watching the progress of the acid among the grains. Does a grain, as the acid reaches it, first swell and then quickly melt away? Do any starch grains survive the action of the acid? Write down the nature of the reaction in your book.

If you should wish to observe the starch grains with the **high-power**, you must be very careful to make sure that no sulphuric acid gets on to the objective as you turn this on. If, by any chance, an acid or an alkali or any other harmful chemical substance should get on to an objective, you must **unscrew the objective, dip its end in water**, and wipe it dry.

Other strong acids act on starch grains like sulphuric acid.

Reaction No. 4. Heat some Starch Grains in Water.—To do this, put a fairly large drop of water on a slide. Add plenty of starch grains, a small drop of iodine to colour them, **but no cover-glass**. Then heat the drop over a spirit lamp until the water just boils. Try to do this without breaking the slide. The flame must be under the water.

Now put the slide **on the table** and add a cover-glass. Let the slide **cool** for a minute or two. Run a little more water under the cover-glass if the drop has dried out too much.

Examine the preparation under the **low-power** of the microscope. If the heat was properly applied, you will find that many of the starch grains have become **greatly swollen**. Compare their size with that of unswollen grains. The swollen grains may look bluish owing to the presence of the iodine. Use the **high-power**, if you so desire.

Reaction No. 5. Add Iodine to Starch Paste.—Common paste used by bill-stickers, paper-hangers, book-binders, etc., is made by mixing about half a pound of wheat-flour with a quart of cold water. A little powdered alum is then put in, or, if used by book-binders, a little powdered resin, which adds to its stickiness. The mixture is then boiled, care being taken to keep it from burning, until it is of the right thickness. As the heat is applied, the wheat starch grains swell up, lose their identities, and melt into one another.

Some Potato starch paste has been made in the laboratory by heating Potato starch in boiling water. Some of it is in a test-tube supplied. The paste is a colloidal solution. Observe it macroscopically. Note its characteristic **opalescence**.

To the starch paste in the test-tube add a drop or two of

iodine. Does the paste give the reaction characteristic of starch ? Record your answer.

C. A STUDY OF THE PULP-CELLS OF A BANANA FRUIT

Musa sapientum, the Banana, with its sub-species *M. paradisiaca*, the Plantain or Pisang, is one of the most important food-plants in existence, and is everywhere cultivated in the tropics where it yields 40 times as much food per acre as even the potato. One or more living specimens may be seen in the greenhouse at Assiniboine Park (compare living plant exhibited, or a drawing on the blackboard).

The Banana belongs to the monocotyledonous tropical family **Musaceae**. It is a gigantic herb (18–20 feet high) and not a tree as one might at first sight suppose. Its leaves spring from a short stem which is mostly underground (a rhizome). The basal parts of the leaf-stalks, the **leaf-sheaths**, are rolled round one another and form a structure which looks like an aerial stem and attains a height of many feet. Each leaf, in addition to its long stalk or **petiole**, has a very large oval emerald-green blade or **lamina**. The lamina has a stout **mid-rib**, and **parallel veins** (vascular bundles) run from the mid-rib to the leaf-margin. The edge of the leaf is easily torn between the veins, as these are not netted in the same way as in a Dicotyledon. The rain and wind soon reduce a leaf to a very ragged condition. The fruit grows in great bunches which hang down between the leaves and often weigh as much as 100 lbs. each.

The fruit of the Banana is an elongated **berry**. Its **pericarp** consists of two layers: a tough **epicarp** (the skin) on the outside, and a soft and succulent **endocarp** within. When the ovary, which matures into a fruit, is young, it has **3 chambers** (loculi) in it, as in the pistil (gynaeceum) of a Narcissus or Daffodil flower, and each chamber contains many **ovules** (potential seeds) attached to the wall near the centre of the ovary (axillary placentation). Compare the diagram provided or on the blackboard. However, none of the fairly numerous ovules in the ovary of a cultivated Banana plant ever mature and develop properly into seeds; but, instead, they become shrivelled and blackened. We can then call them **aborted seeds**. Look for some of these aborted seeds in the piece of fruit provided.

As the fruit ripens, the endocarp becomes succulent and its

pulpy substance grows inwards toward the aborted seeds. The three chambers originally present in the ovary soon become filled up by this ingrowth, and thus the fruit comes to have the appearance which you can now observe.

The fruit of the cultivated Banana, as we have seen, is **seedless**. The Banana plant, therefore, **cannot be propagated from seeds**. New plants are always obtained by growers from pieces of the underground stem (**cuttings**). Wild Banana species, on the other hand, have large seeds, and their endocarp is only slightly pulpy. No doubt, when Banana plants were first cultivated by primitive man, the fruits contained seeds. The seedless condition must have come into existence during cultivation many centuries ago. The absence of seeds in the Banana fruits sold in shops accounts in part for the popularity of bananas. So far as children are concerned, there is no danger of their swallowing any "stones."

Make a sketch of a cross-section of the fruit supplied to you. The fruit was greenish and unripe. A piece of ripe fruit will be studied later. In your drawing indicate the **pericarp** (note the numerous **vascular bundles** or conducting strands in it), the **pulpy endocarp**, and the **aborted seeds**; and represent what can still be seen of the outlines of the three chambers now filled with pulp.

Examination of the Pulp of a Green or Unripe Banana Fruit.—With a knife or scalpel remove a very little pulp from the fruit, place it on a slide, add a drop of water to it, and with the help of two needles (or a knife and a needle) **tease the cells of which it is composed apart**. Then add a cover-glass and examine first with the low-power objective and then with the high.

Note that the **pulp is made up of cells** which are very easily separated from one another. This in part accounts for the digestibility of bananas. When the pulp is very young, the cells are all firmly attached to one another by the middle lamella of their cell-walls; but, when the fruit begins to ripen, the middle lamella breaks down and to a large extent disappears; hence the ready separation of the cells which you can now observe. Note the extent to which the individual cells vary in shape.

With the high-power objective **study the structure of a few cells in detail**. Each cell consists of a thin **cell-wall** (made of cellulose), a **layer of cytoplasm** lining the interior of the cell-wall, a nucleus **embedded** in the **cytoplasm** (not likely to be seen in most cells, but may be looked for), a **vacuole** (a large space

enclosed by the cytoplasm) filled with **cell-sap**, and a number of **large white granules**. The granules are in the vacuole. What are the granules? Are they aleurone grains or starch grains? Examine their structure. Are the grains **laminated** like a starch grain or **non-laminated** like an aleurone grain?

Aleurone grains are made up of proteins, and proteins turn **yellow** when acted upon by iodine, whereas starch grains ($C_6H_{10}O_5$)_n turn **blue** with iodine. Add iodine in the manner already described and thus decide the nature of the grains microchemically.

The iodine is poisonous and kills the protoplasm (cytoplasm and nucleus) in the cells and, since protoplasm is made up chiefly of proteins, the dead protoplasm turns yellow with iodine. The cytoplasm, on being killed, contracts away from the cell-wall. Look for protoplasm in a few of the cells.

Make a drawing of a few cells as seen with the high-power objective. As usual, write above your drawing what it represents and name the parts: **cell-wall, cytoplasm, starch grains**; also the **vacuole** and **nucleus**, if you should be able to observe them. The largest cells should be made about two inches long. This will allow you to show the shapes and structural details of the starch grains.

Before Leaving your Seat.—Turn on the **low-power** objective. Then **remove** any slide from the stage. Place dirty slides with their cover-glasses in the saucers. Place clean slides on your table number. Put lens paper, blotting-paper, and piece of cheese-cloth in the Botany drawer (marked "B").

CHAPTER VI

THE PULP-CELLS OF A RIPE BANANA FRUIT. THE ACTION OF DIASTASE ON STARCH. THE CELLS OF A COTYLEDON OF A PEA EMBRYO

Materials and reagents supplied:

- (1) A yellow-green unripe Banana fruit;
- (2) A blackish-yellow fully ripe Banana fruit;
- (3) A moist Pea seed from which sections are to be cut with your own razor;

- (4) Tannic acid crystals ($C_{14}H_{10}O_9$) ;
- (5) Ferric chloride (Fe_2Cl_6) ;
- (6) A test-tube containing diastase (in a malt extract) ;
- (7) A test-tube containing Potato starch paste ;
- (8) An empty test-tube ;
- (9) Iodine, which colours starch blue and proteins yellow.

A. A STUDY OF THE PULP-CELLS OF A BANANA FRUIT (*continued*)

Examination of the Pulp of a Blackish-yellow Fully Ripe Banana Fruit.—Some nearly ripe bananas with yellow skins have been kept in a very warm place for three days, with the result that they have become fully ripe. Their skins are now blackish, thinner, and much less tough, and their pulp has become very soft indeed.

Make a microscopic **comparison of the pulp of a fully ripe banana** (a piece is provided) **with the pulp of an unripe banana** (already studied). In what essential point do the cell-contents of the two kinds of pulp differ from one another ? Decide this for yourself.

If the **starch** has disappeared or almost disappeared from the cells of the fully ripe banana pulp-cells, what made it disappear and what has become of it ? The answer is as follows : The pulp-cells of a ripening banana fruit contain in solution in the cell-sap an organic chemical substance known as **diastase**, and in the presence of this substance the insoluble solid starch grains become hydrolised and converted into soluble **sugar**. Sugar in solution cannot be seen with the microscope. Diastase, like pepsin, belongs to a class of chemical substances known as **enzymes** (organic catalysts).

An **unripe banana** is green, without an attractive aroma, its pulp is relatively solid, it lacks sweetness, and is astringent to the taste. The lack of sweetness is due to the fact that all the **pulp-cells are filled with starch grains** and contain little or no sugar. The astringency is due to the presence of **tannin**.

A **fully ripe banana** is black or blackish-yellow, has an attractive aroma, its pulp is relatively soft (owing to the disappearance of the middle lamellae of the cell-walls), it is very sweet to the taste, and it is no longer astringent. The sweetness is due to the fact that all the pulp-cells, owing to the action of diastase, have lost their starch and now **contain an abundance of sugar**. The

disappearance of the astringency is due to the tannin having become fixed in jelly contained in certain cells to be studied later.

Bananas are usually eaten before they are fully ripe and while they still have yellow skins. The pulp cells in such so-called **ripe** bananas contain a good deal of sugar, but also some starch grains. When a not-fully-ripe banana has been eaten and is undergoing digestion, all the starch grains left in the pulp-cells are converted rapidly into sugar by diastase produced in the stomach.

Make a sketch of some pulp-cells from a fully ripe banana fruit. While studying individual cells in preparation for drawing them, look for the **nucleus**. Now that the starch grains have disappeared, it may be possible for you to find a nucleus embedded somewhere in the shrunken layer of cytoplasm.

Is there any tannin in a Banana fruit? Tannins are astringent substances which interfere with digestion. They form 10 per cent. of dry tea leaves and are extracted from the bark of Oak trees for tanning leather. Black ink is commonly made by combining tannin with a substance containing **iron**.

A test for tannin. Put some tannic acid crystals on a slide and add to them a drop of **ferric chloride** (an iron salt, Fe_2Cl_6). A blackish ink is produced, the characteristic reaction for a tannin. Record this reaction in your book.

Tannin in a banana. Slice away the surface layer from a piece of unripe banana, and spread a few drops of ferric chloride over the newly exposed surface. After a minute or so, can you observe any blackish strings or patches in the pericarp or in the pulp? If you can, the inky reaction must have taken place and some tannin must be present in the banana.

With a knife or scalpel, remove some little bits of blackened pulp to a slide, add a drop of water, tease the cells sufficiently apart so as to spread them in the drop, and mount the preparation with a cover-glass in the usual way. Examine the preparation with the microscope and find the **tannin cells**. These cells are **much larger** than the pulp-cells; they are **barrel-shaped** and **occur in chains**, and they are distinguished from all the other cells by their **blue-black colour**. They store up tannin in their cell-contents and do not contain any starch. The astringency of an unripe banana is due to the tannin in the tannin cells. As a fruit ripens, the contents of the tannin cells become gelatinous. The jelly fixes the tannin and largely prevents its

escape. Hence, though there is tannin in a ripe banana, the fruit is not astringent to the taste.

Sketch some of the tannin cells with a few pulp-cells by their side, to indicate their relative sizes. Indicate the **cell-wall** and the gelatinous cell-content or jelly in which the **tannin** is held.

B. AN EXPERIMENT IN WHICH STARCH DISAPPEARS IN THE PRESENCE OF DIASTASE .

If **malt** (dried germinating barley grains) is crushed up into powder and water is added, **diastase** comes out of the malt into the water. A filtered watery extract of malt therefore contains diastase. Such an extract has been prepared by one of the demonstrators.

You will be supplied with :

- (1) a test-tube containing diastase (in a malt extract),
- (2) a test-tube containing Potato starch paste,
- (3) an empty test-tube,
- (4) iodine in solution.

Pour half the starch paste into the empty test-tube. Keep one of these test-tubes as a **control** and use the other one for the **experiment**.

Add to the starch paste in the experimental tube two or three drops of the diastase-containing malt extract. Shake up, and then let the diastase act on the starch for about five minutes.

After the diastase has been acting for about five minutes, add two or three drops of **iodine** first to the starch paste in the **control tube**, to see whether or not starch is really present in it. Then add two or three drops of iodine to the **experimental tube**. If the diastase was effective, the contents **of the experimental tube will not turn blue**, and we can conclude that **the starch has disappeared owing to the action of the diastase**. If the starch has disappeared, it has all been converted into **sugar**. This could be proved by using a reagent known as **Fehling's solution**, but it is not convenient to make the test to-day.

Record the effect of adding diastase to Potato starch paste in your book.

C. A STUDY OF THE CELLS OF SEEDS AND OF THE RESERVE FOOD MATERIALS STORED WITHIN THEM

Sections and Section-cutting.—Seeds, like all other structures of Flowering Plants, are made up of **cells**. In order to observe

the cells of seeds it is necessary to prepare **thin sections** (slices) of the tissues which one desires to study. Sections can be cut either with a **microtome** or with a **hand-razor**.

The **microtome** is an elaborate and somewhat costly instrument which works on the principle of the guillotine. By means of it one can cut a piece of root, stem, leaf, etc., into a very large number of serial sections of any desired thickness. It is possible to cut a single cell, and even a single nucleus, into several distinct sections. Hence the microtome is much used by research workers in the study of the structure of plants and animals.

Usually the material to be cut with a microtome is first **killed** and the protoplasm in its cells **fixed** in the position it had in nature by means of some fixing solution, e.g. chrom-acetic acid. The material is then dehydrated gradually with **alcohol**, and then passed through **xylol** into melted **paraffin wax**. The wax is allowed to cool and harden, and then the block of wax with the piece of material embedded within it is **cut** with the microtome knife **into thin sections** (about 10 microns—0.01 mm. thick). A paraffin section so cut is then laid on a slide previously smeared with albumen (to cause the section to adhere to the slide), and the slide is warmed to melt the wax. The wax is removed by xylol, and the xylol by alcohol. Then the section can be stained with an aniline dye or some other pigment. After the section has been stained it is washed with alcohol (to dehydrate it), and then with xylol (to remove the alcohol), and then it is mounted in a drop of **Canada balsam** (obtained from the resin-ducts in the wood and bark of *Abies balsamea*—the Balsam Fir of our forests). The balsam slowly dries up and hardens. We thus obtain a **permanent preparation** which may be kept for years. Sections of stems, roots, etc., cut with the microtome and permanently mounted in Canada balsam will be provided for study later on in the session.

The **hand-razor** is a most useful instrument for rapidly cutting sections of seeds, stems, roots, etc. Whereas **with the microtome method** the material cut is always dead and the various operations of fixing, embedding in paraffin, cutting and staining, take a week or ten days to carry through, **by employing a hand-razor** one can cut sections not only of dead but also of living material and, with a little practice and skill, one can cut good sections from the material in less than one minute. It is impracticable to use a microtome in this class, and the hand-razor is far better

suited to our purposes. You must therefore **master the technique of using the hand-razor**. Skill comes with practice and determination to get good results. Before sections can be cut it is necessary to **prepare the material** with a pen-knife or scalpel so that it can be held easily and firmly.

D. A TRANSVERSE SECTION OF A COTYLEDON OF A PEA EMBRYO

To Prepare the Material for Section Cutting.—Some peas were soaked for a few hours to soften them. Some of these seeds have been prepared for your use.

Take a pea. Remove the testa. Break off one of the cotyledons from the embryo. Lay the cotyledon on the table on its flat side and then, with a pen-knife or scalpel (**not** the razor), cut away pieces above and below and at its sides until you have left a brick-shaped block (see diagram on blackboard) that can readily be held between a finger and thumb. The block of tissue should be as long as possible, but the end from which the sections are to be cut should be only about 2×3 mm. A larger cutting surface is not advisable, as what we require is not a large section but a **small section thin enough to allow us to observe the individual cells well**; and it is easier to cut a small section well than a large one.

Kind of Razor Required.—Safety razors (even those in metal knife-handles), unfortunately, are unsuitable for cutting good hand-sections of plant tissues. It is therefore necessary for you to use one of the old-fashioned **hollow-ground razors**.

You must provide yourself with a **hollow-ground razor** and **bring this razor to all subsequent practical classes** (unless you are otherwise instructed). Hollow-ground razors can be purchased in hardware stores at a cost of about \$1.25.

Care of the Razor.—You are reminded that the razor has a very sharp and delicate edge. **It must not be used for rough work**, but solely for cutting sections. Its edge must be dried and cleaned after use, and every care must be taken to keep the razor as sharp as possible. Stropping the razor is an art in itself and, when attempted, must be done carefully. The strop should be smooth and clean, and the edge of the razor must always be drawn over it in such a way as not to cut it. It is **impossible to cut good sections with a blunt razor**.

How to Hold the Razor in the Right Hand.—Turn the handle

back until it sticks out at right angles to the blade. For the moment, hold the razor handle with the left hand so that the handle is directed away from your body. Then grasp the steel part of the razor below the blade with the **first and second fingers** and with the **thumb** of the **right hand**. The two fingers are put right and left of the handle and the thumb in front on the milled surface. By holding the razor in this way, you get a firm grasp of it and it cannot slip when sections are being cut. There are other methods of holding a razor for section cutting, but the one described is recommended as the best. During the process of cutting sections the material is to be held **upright** and the razor should be **held and worked in a plane parallel to the upper surface of the table**.

How to Hold the Material in the Left Hand.—Hold the block of material between the first finger and thumb of the left hand. The block should be held **upright**, as sections are to be cut from the uppermost of the two ends. The finger should be held **horizontally** (parallel to the table edge), as the razor blade is to rest on it, as on a cushion, when in use. The top of the thumb must be held slightly below the front side of the material. Otherwise it may be cut by the razor.

Lubrication of the Razor Blade and Material.—When sections are being cut, they do not slide readily on to the razor blade if the blade and the material are dry. Before any sections are cut, therefore, **the razor blade should be wetted** along its edge with water. The water can best be put on with a finger of the left hand. The material, held in the left hand, should be **dipped in water** just before it is to be cut.

Actual Process of Cutting Sections.—The dipped material being held upright in the left hand in the manner already described, take the razor held in the right hand and rest its wetted blade on the horizontal finger of the left hand and then proceed to cut sections. The razor and finger of the left hand should be parallel to the table and the razor is to be worked in a horizontal plane. **The arms should be perfectly free** and not resting on the table.

The razor should be given a **sliding motion**, much like that given a saw. The razor is drawn **from left to right** and toward you as a section is cut.

The **sections** must be cut **as thin as possible**. By cutting away a series of thin sections from the top of your material you will not injure your razor.

The **first section or two** will serve to level the top of the block of material and may be discarded. When the top is level, cut **two or three more sections** as thin as possible. The thinner the sections, the better. Often it is best to start cutting a section with the **razor blade edge resting a little way over the top of the material**. Sections which are **smaller** than a complete transverse section are usually better than complete sections, as they are apt to be thinner. A piece no larger than 1-2 square mm. is extensive enough, if it is thin.

Transferring the Sections to a Drop of Water on a Slide.—Put a drop of water on a slide. Hold the razor handle in the **left** hand and gently bring the edge of the blade, where the sections are, down to the slide. Then, with the help of a needle held in the **right** hand, pass the sections into the water. Do not injure the razor-blade edge while performing this operation.

Drying the Razor.—Now wipe your razor blade dry with the help of cheese-cloth or a handkerchief. If you wipe toward the edge of the razor (both sides at the same time), you will not cut the cloth. The removal of the moisture on the razor blade prevents its edge beginning to rust and losing its sharpness. When the razor blade is dry, carefully **fold up the razor and put it at the back of the table**.

Washing and Mounting the Sections.—As the sections were being cut with the razor, many of the cells were cut in two, thus allowing their cell-contents to escape. You will notice that the drop of water around the sections on the slide is somewhat milky-looking. Stir the sections with a needle and this milkiess will become still more apparent. The **milkiess** is due to **starch grains, aleurone grains, and protoplasm** which have escaped from broken cells.

Wash the sections free from escaped cell-contents by adding more water to the slide and then running off the excess water into your saucer. Run more water down the slide and over the sections until they are clean. A needle should be used for stirring the sections and preventing them from being washed away.

When the sections (one, two, or three) have been cleaned, **remove all excess water** from the slide by means of blotting-paper, cover the sections with a **fresh drop of water**, and then add a **cover-glass**.

Examination of the Sections.—Explore your preparation with the low-power objective and find the best section for study.

In any section which is thin, some of the cells will appear to be more or less **empty** on account of their having been cut open by the razor and having lost their contents. The intact cells will be seen to be filled with dense cell-contents.

Make out the **cell-walls**, some **intercellular spaces**, **starch grains**, and **aleurone grains**. Use the high-power objective only after you have made out as many points as possible with the low-power.

Note the thickness of the **cell-walls** relatively to the width of the cells. The cell-walls, as is usual in plants, are made up of cellulose $(C_6H_{10}O_5)_m$. The **intercellular spaces** (air-spaces) are more or less **triangular** where three cells meet together, but are more or less **four-sided** where four cells meet together. In nature, the intercellular spaces are filled with **air** and, if there is still any air in any of the spaces, these spaces will look dark like air-bubbles. In cutting the sections, however, it often happens that the cell-contents of broken cells **are smeared into many of the intercellular spaces** by the razor. If, therefore, you find aleurone grains held in protoplasm occupying some of the triangular or four-sided intercellular spaces, remember that this is due to accident.

Each cell contains **protoplasm** (cytoplasm and a nucleus). The nucleus, owing to the density of the reserve food materials, can be found only with the help of stains, and we are therefore not likely to see it now. The **starch grains** and **aleurone grains** are embedded in the cytoplasm like plums and currants in a plum-pudding.

The **starch grains** are the largest granules in the cells and the **aleurone grains** are very much smaller. Under natural conditions both kinds of grains are **colourless**.

In some varieties of Pea the starch grains are **simple**, in other varieties **compound**. A compound grain is made up of several grains massed together so that at first, collectively, they look like one simple grain. Are your grains simple or compound?

The Differentiation of Starch Grains and Aleurone Grains by Means of Iodine.—Turn on the low-power objective. Slide your slide off the stage and put it on the table. Deposit a drop of **iodine** on one side of the cover-glass and join it to the edge of the cover-glass with a needle. Some of the iodine will be drawn

under the cover-glass. **Suck more iodine** under the cover-glass by means of blotting-paper until the iodine comes to one or more of the sections.

Note with the microscope that, as the iodine invades a section, the cell-contents react to it. The **starch grains** turn **blue** and then gradually **blue-black**. The **aleurone grains**, more slowly, turn a distinct **yellow**. Also notice that the **cell-walls** turn **yellow** with iodine.

If the iodine has not penetrated into the sections sufficiently, you can remedy this as follows: Turn on low-power objective. Slide the slide off the stage and put it on the table. **Lift the cover-glass up and down a time or two**. This will allow the iodine to get to each section from above, and all the cells will soon show the effects of the reagent.

The **aleurone grains** can now readily be seen as small but very numerous **yellow particles**. They are prevented from falling away from one another by the cytoplasm in which they are embedded. The **cytoplasm** is made up chiefly of proteins and, therefore, like the proteinaceous aleurone grains, turns **yellow** with iodine. We thus have deep yellow aleurone grains held in a lighter yellow matrix of cytoplasm.

The **starch** stored up in the cotyledons constitutes the **carbonaceous** reserve food material and the **aleurone** grains the **nitrogenous**. No oil is present, and the cell-walls are moderately thin and not greatly thickened by layers of reserve cellulose (hemi-cellulose). When a Pea seed germinates, the **solid starch** is converted into **soluble and diffusible sugar** by means of diastase; while the solid aleurone grains are converted into **soluble and diffusible nitrogenous compounds** by means of a proteolytic enzyme. The sugar and nitrogenous compounds pass in solution to the radicle and plumule, and thus provide for their growth into a root and a shoot respectively. Whilst the embryo is developing into a Pea seedling, the two cotyledons become exhausted of their reserve food materials. They shrivel, die, decay, and thus finally disappear, while the seedling they nourished grows into a mature plant.

Make a drawing showing a few of the cells of a cotyledon. The largest cell should be about 1.5 inches wide. Each cell-wall is thick enough to be put in by two parallel lines. Indicate the **cell-walls, intercellular spaces, starch grains, and aleurone grains**, the two last embedded in **cytoplasm**.

Record in your book the reactions of the cell-walls and the cell-contents to iodine.

Before Leaving Your Seat.—Remember to turn on the **low-power** objective and to **remove any slide** that may be on the stage. Put dirty slides in the saucer and clean ones on your table number. Put cheese-cloth, blotting-paper, and lens paper in your drawer marked “B.” See that your razor is perfectly dry and clean. Clean it with water if necessary.

CHAPTER VII

A STUDY OF THE CELLS OF A CASTOR OIL SEED AND OF A PEA-NUT SEED, AND OF THE RESERVE FOOD MATERIALS STORED WITHIN THEM

Materials and reagents supplied :

- (1) A Castor Oil seed ;
- (2) A Pea-nut seed ;
- (3) Water, which does not mix with castor oil ;
- (4) Iodine, which stains starch grains blue and aleurone grains yellow ;
- (5) Sudan III, which stains oil a brilliant orange-red ;
- (6) Alcohol, 95 per cent., which mixes with castor oil and can therefore be used to remove it ;
- (7) Caustic potash, 10 per cent., which dissolves the proteins making up the matrix and the crystalloids of aleurone grains ;
- (8) A dilute acid (hydrochloric or acetic), which dissolves the globoids of aleurone grains.

A. A TRANSVERSE SECTION THROUGH THE ENDOSPERM OF A CASTOR OIL SEED

To Prepare the Material for Section Cutting.—Take a Castor Oil seed. Remove the testa. With a pen-knife or scalpel **cut off a piece** from one end of the endosperm and thus make a flat surface from which sections may be cut.

The endosperm is very **oily**. It is therefore **unnecessary** and **inadvisable** to lubricate the razor or material with water.

Cutting the Sections.—With the dry razor cut **some transverse sections** from the prepared endosperm surface. The instructions (except in respect to lubrication) already given for cutting sections from a piece of Pea cotyledon apply equally here. Start cutting with your **razor-edge on the top of the material**. The sections must be made **as thin as possible**. A small thin bit (1–2 square mm., or even less) is far better than a large thick section. Transfer the sections from the edge of the razor to a dry slide with the help of a needle.

Mount the section or sections **in water** under a cover-glass in the usual way, and examine the preparation with the microscope. Low-power first, then the high-power.

Study of the Oil.—The cells are rather small and their walls are very thin. In the thinnest part of your best section you should just be able to make out the outlines of the cells. The **cell-contents are very dense**. This is due to the fact that the protoplasm in each cell contains a large amount of **oil** and also a considerable number of **aleurone grains**, some of which are very large. The oily cell-contents make it impossible to see the cell-walls clearly.

Remove the slide from the stage (turn on low-power objective first: never forget this rule), place it on the table, and **press the cover-glass down on the section** with the wooden handle of your needle or with a knife or scalpel. Watch the section, and do not break the cover-glass in this operation. The **oily cell-contents** will be **pressed out of some of the cells** beyond the margin of the section. With the microscope observe these oily-looking contents. With the high-power you will be able to see **oily streaks** on the under side of the cover-glass, and also large rounded oily drops in the water at the edge of the section.

Add a drop of **iodine** to the preparation (do the work, as usual, on the table). **Do any of the cell-contents turn blue?** If they do, starch must be present; if they do not, starch must be absent. Record in your book whether starch is present or absent.

Make another preparation and observe the effect upon it of the staining reagent **Sudan III**. Cut the section dry as before and put it on a slide with a needle. **Add a drop of Sudan III**, and then a cover-glass. Observe with the microscope.

Sudan III colours oil a **brilliant orange-red**. As tested by this

reagent, is or is not oil present in the endosperm? Are the aleurone grains coloured? Record your answers.

Study of the Aleurone Grains.—Up to the present, the aleurone grains have been largely hidden from view, owing to the presence of great quantities of oil in the protoplasm. To see the aleurone grains clearly, it is necessary to get rid of the oil. Now **castor oil is miscible with absolute alcohol** and even with 95 per cent. alcohol. We shall therefore use alcohol for **clearing the sections of oil**.

With a dry razor, cut two or three small very thin sections. With a needle transfer them to a dry slide. Hold the slide over the floor and then pour on to the sections a **large drop of 95 per cent. alcohol**. Stir them about with a needle so that the alcohol which bathes them will have a better chance to dissolve out their oil. After about half a minute or longer, run the alcohol off into a saucer and renew the bath once more. Now **remove** as much of the **alcohol** from the slide as you can with the help of cheese-cloth or blotting-paper. Add to the sections a **drop of iodine**, immerse the sections in the iodine and **stir them with a needle**, and then **add a cover-glass**. Examine the preparation first with the low-power to find the best section, and then with the high-power to observe the details.

The **aleurone grains**, which are much larger than those of the Pea and Wheat (they are usually large in oily seeds), will now be well seen. They turn **yellow** with iodine, thus revealing their protein nature. There are a number of large aleurone grains in each cell. The **walls of the cell** and the granular **cytoplasm** (coloured light yellow) can now be readily observed.

Having found the best place in your section, endeavour to make out the **details of the structure of the aleurone grains**. The aleurone grains of the Castor Oil endosperm are not only much larger, but are more complicated than those in the cotyledons of a Pea embryo.

A single large aleurone grain consists of an oval proteinaceous **matrix** (ground substance) in which are embedded one or more protein-crystals called **crystalloids** and one or more rounded bodies called **globoids**. The matrix turns **yellow** with iodine and the crystalloid **deep yellow** or **brown**, while the globoid remains **white**.

All the parts of an aleurone grain were formed from cell-sap in a vacuole in the cytoplasm of a cell as the seed ripened. The

least soluble material, a double phosphate of calcium and magnesium, was precipitated out first to form the **globoid** or **globoids** (inorganic matter). The next thing to solidify was the **crystalloid** (one or more). Finally, the general **matrix** (ground-substance) of the grain, the most soluble part of the grain, solidified as an amorphous mass and thus came to enclose the crystalloid and globoid which had been formed earlier. Crystalloids differ from ordinary crystals of salt, etc., in that they are of colloidal origin and **swell** up in glacial acetic acid, etc.

Drawing.—Make a drawing showing a few cells of the endosperm. Each cell should be made an inch or more long. Show the **cell-walls**, **intercellular spaces**, the **cytoplasm** containing oil (oily cytoplasm), and the **aleurone grains**. As usual, write above your drawing what it is meant to represent. The **cell-walls** should be drawn with a **single line** each, as they are so very thin.

Draw a few aleurone grains on a larger scale by themselves (about $\frac{2}{3}$ inch long or more). In a single grain indicate the general **matrix**, the **crystalloid** (one or more if you see them), and the **globoid** or **globoids**.

A Microchemical Method for Removing All the Aleurone Grains from the Cells by Successive Stages.—By using certain reagents in succession, we can first remove the **oil** from the cells, then part of the **matrix** of the aleurone grains, then the rest of the matrix and the **crystalloids**, and finally the **globoids**. Proceed as follows :

(1) Cut two or three small thin sections with a dry razor and transfer them to a dry slide.

(2) Bathe the sections in two successive baths of 95 per cent. alcohol. **This removes the oil** and clears the sections for further operations.

(3) Wipe away all the alcohol you can and then add a **drop of water**. Stir the sections in the water with a needle to allow the water to take the place of the alcohol. Add a cover-glass. Observe a section under the microscope. You will find that the **matrix** of the aleurone grains becomes or has become **more transparent**. This is due to the fact that part of it **goes into solution in the water**.

(4) Put slide on table, remove cover-glass, remove most of water, add a **drop of iodine**, stir section in iodine with a needle, add the cover-glass, and observe under the microscope. The three parts of each large aleurone grain will now readily be seen.

The crystalloids will be brown, the globoids colourless, and the matrix yellowish. The colour will be helpful in the next operation.

(5) Remove slide to table. Put a drop of **10 per cent. caustic potash** at edge of cover-glass and suck it toward the sections by means of blotting-paper, until the solution just reaches the first of them. Observe a section **as the potash invades it**. It becomes colourless. Observe the **junction zone**. With the high-power you should see the rest of the matrix and the brown **crystalloid** (or crystalloids) in each large aleurone grain **go into solution and disappear, leaving a large globoid or several small globoids behind as spherical colourless bodies**. The loss of the yellow colour in a section indicates how far the reagent has penetrated, and it is at the **junction zone** that you can see best how the potash acts upon the aleurone grains.

In the sections, there are now left the cell-walls, part of the granular cytoplasm, and the **globoids**. We have now to remove the globoids, the last remnants of the aleurone grains.

(6) Remove slide to table. Lift up cover-glass. Place a fresh drop of **water** on the slide and transfer section to it. Stir it about to **remove the caustic potash**. Take **another slide**. Put two small drops of water on it near together. Transfer the section to one of them, stir it round, and then put it in the other drop. Remove first drop with blotting-paper. The section has now been well washed. Add a cover-glass. Look at the preparation with the microscope to see that the **globoids are still present**.

(7) Remove slide to table. Put a **drop of dilute acid** (dilute hydrochloric, or acetic acid) at the edge of the cover-glass and, with blotting-paper, suck it under the cover-glass until it reaches the sections. Observe the **junction zone** with the microscope. With the high-power you should be able to **see the globoids diminishing in size and disappearing one by one**.

The cells have now **lost their oil** (if your section was properly cleared) **and their aleurone grains** in their entirety, and we have left only the cell-walls and traces of the cytoplasm. It would be possible to dissolve away the cellulose of the cell-walls with cupric ammonia, but this will not be attempted to-day.

B. THE RESERVE FOOD MATERIALS IN A PEA-NUT SEED

A Pea-nut seed is provided. **What reserve food materials are stored up in its cotyledons?** Solve this problem with the

help of the methods with which you are now familiar. Report the results of your investigation in your book.

Before Leaving Your Seat.—Remember to turn on the **low-power** objective, and to **remove any slide** that may be on the stage. Put dirty slides in the saucer and clean ones on your table number. Put cheese-cloth, blotting-paper, and lens paper in your drawer marked “B.” See that your razor is perfectly **dry** and **clean**. Clean it with water (rub with a finger) if necessary.

CHAPTER VIII

A STUDY OF THE CELLS OF A GRAIN OF WHEAT AND OF A COFFEE SEED, AND OF THE RESERVE FOOD MATERIALS STORED WITHIN THEM

Materials and reagents supplied :

- (1) A grain of Wheat, soaked in water for 12 hours and then dried for 2 hours ;
- (2) A Coffee seed, soaked in water for 12–14 hours ;
- (3) Iodine, which stains starch grains blue and aleurone grains yellow ;
- (4) Sudan III, which stains oil a brilliant orange-red ;
- (5) A slide showing crystals of caffeine obtained by micro-sublimation may be passed around.

A. THE MICROSCOPIC STRUCTURE OF A GRAIN OF WHEAT (*Triticum vulgare*)

Preparation of Material.—Dry wheat grains are too hard and brittle to be cut with a razor, and fully soaked grains are too soft. The grains supplied to you were soaked in running water over-night (about 12 hours), were dried on their exterior with a cloth in the morning, and were then left exposed to the drying air of the laboratory for about two hours. The grains now contain just the right amount of moisture to permit of good sections being cut from them without injury to the razor.

Macroscopic Observation of a Grain.—Examine one of the grains of wheat supplied. Once more notice : (1) The more

flattened side with its groove (crease), (2) the more rounded bulging side, (3) the hairy apical end, and (4) the basal end with the impression of the tiny embryo clearly showing in the red membrane which covers the whole grain and consists of pericarp and testa combined.

To obtain a Suitable Surface for Section Cutting.—Lay a grain of wheat on the table on its flatter grooved side. Then take a sharp pen-knife or scalpel and cut off the end of the grain just above the embryo. Cut vertically downwards toward the table so as to cut the grain in a transverse direction.

Look at the surface of section just made and notice: (1) the depth of the groove, (2) the membrane which surrounds the grain, protects it, and gives it its red colour, and (3) the whitish inner endosperm or starchy endosperm which occupies all the interior part of the grain. Make a **sketch** of this transverse section in your book. This should not take more than about a minute. Make the drawing about an inch wide and indicate the groove, exterior membrane, and starchy endosperm.

Section Cutting.—Take the grain in your left hand and hold it upright between your thumb and finger, with the flatter grooved side toward your thumb. The first finger should be held horizontally and parallel to the table edge so as to form a cushion for the razor.

Cut the sections **dry**. Do not lubricate either the material or the razor with water, as without such lubrication one can obtain better results.

Holding the razor in the right hand in the manner already described in Chapter VI, cut some thin sections from the prepared surface of the grain. Try to obtain a thin section through the pericarp, testa, aleurone layer and part of the starchy endosperm. A **complete transverse section is not required**. A small thin incomplete piece, including only portions of the layers mentioned, will show all the structures you are to study and is easier to cut properly than a complete section.

Mounting the Sections.—When the sections have been cut, take the razor in the left hand and, with a needle held in the right, transfer the sections to the middle of the **dry slide**. Then put a drop of water near the sections and with a needle put the sections into the drop, but **do not stir** them about. Then add a cover-glass and study the preparation with the microscope.

Examination of the Sections.—With the low-power objective

find the best section and then the **best part of the best section**. Make out as many points as you can with the low-power, and then use the high-power. The following structures can be readily seen in a good section :

(1) The **pericarp** (fruit-coat, corresponding to the pod of a Pea fruit), made up of several layers (about 4) of thick-walled empty cells. From these cells the protoplasm has disappeared and, in **dry** grains, the cell-lumina (spaces) contain air only. In your water-mounted preparation water will have taken the place of the air. The walls of the cells are very tough and well fitted for protecting the grain mechanically.

(2) The **testa** (seed-coat, corresponding to the testa of a Pea seed) consists of two layers of cells : (a) a thin distinctly **red layer** just under the innermost layer of cells of the pericarp, and (b) a somewhat thicker **white layer** just beneath the red one. Both the red and the white layers consist almost entirely of cell-walls ; the cells have been crushed (as the grain expanded) and flattened, so that their cell-lumina (spaces) have practically disappeared. There are **traces of the lumina** along the central line of the white layer in the form of streaks. These streaks represent the cell-contents still left when the cell-lumina were crushed out of existence.

The **red colour** of a wheat grain, as you can realise from observing your section, resides in the outer layer of the testa and nowhere else. The red colouring matter has no food value. However, wheat buyers in England are accustomed to buy red wheat from Canada, so farmers find it more advantageous commercially to grow red varieties of wheat rather than white. The Marquis variety of wheat you are now studying has a good red colour from the trader's point of view ; but the colour, you observe, is only skin deep and does not involve the interior of the grain.

(3) The **outer endosperm** or **aleurone layer**. Under the testa is a layer of moderately large square-looking cells which have fairly thick whitish walls without any intercellular spaces between them. This layer is the outermost part of the endosperm and completely surrounds the inner or starchy endosperm. It is usually called the **aleurone layer**, because it contains numerous **aleurone grains**. These hide the cytoplasm (in which they are embedded) and also the nucleus.

The **aleurone layer** is of considerable importance, as may be realised from the following considerations. (a) The aleurone

layer remains intact during the germination of the grain at a time when the inner starchy endosperm is breaking down and its starch is being converted into sugar; so that the aleurone layer protects the inner endosperm from the inroads of bacteria and fungi. (b) When the grain is germinating, the aleurone layer excretes an abundance of the enzyme **diastase** which diffuses into the starchy endosperm and there (along with other diastase excreted by the epidermis of the cotyledon) converts the starch grains into sugar. The sugar is absorbed by the cotyledon and conducted to the radicle and plumule where it is used for growth. (c) The aleurone layer contains a **vitamine** (known as **water-soluble B**) which promotes the growth of animals, including human beings. In making white flour, the miller removes the aleurone layer, the testa, and pericarp as bran, and makes his flour by grinding up the starchy endosperm only. White flour, therefore, does not contain the vitamine just described. Another vitamine (known as **fat-soluble A**) is present in the embryo, but the embryo is removed along with the bran before white flour is made. White flour therefore lacks the vitamins which are naturally present in wheat grains and which are present in whole-meal flour.

(4) The **inner endosperm** or **starchy endosperm**. This makes up the whole of the interior of the grain and, at its base, it is in direct contact with the broad cotyledon of the embryo. The cells have very **thin cell-walls** and are usually longer than the cells of the aleurone layer. There is an **intercellular space** where three cells meet. Each cell contains a nucleus and cytoplasm. **In the cytoplasm numerous rounded starch grains are embedded**, so that the nucleus is completely hidden and the cytoplasm can only be distinguished with difficulty.

Notice that the **starch grains** are **almost spherical**, thus differing in shape from those in a Potato. The starch grains in general fall into two groups: **large** ones which are less numerous, and **small** ones which are more numerous and packed in the interstices (spaces) between the large grains. Owing to the starch grains being of two sizes, the packing of each cell with starch is effectively carried out. To observe isolated starch grains, look for them in the water outside the section.

The cytoplasm contains no aleurone grains, but is rich in **proteins**. Among these proteins are **gliadin** and **glutenin** which together when wetted form **gluten**. You may find strings of

sticky gluten on the outside of your section where one or more of the inner endosperm cells has been drawn over the glass in mounting the section.

Differentiation of the Outer and Inner Endosperm by Means of Iodine.—Run a drop of iodine under the cover-glass and suck it toward the sections by means of blotting-paper. See that sufficient iodine reaches the sections, and then observe the preparation first with the low-power of the microscope and afterwards with the high-power. (If your preparation is unsatisfactory, do not hesitate to make a new one. A new section should be put on the slide, then put in a very tiny drop of water, then a drop of iodine added, and then the cover-glass.)

Notice that the **granular contents of the aleurone layer turn yellow** and not blue, thus showing that they consist of aleurone grains without any admixture of starch grains. Also notice that all the **starch grains**—big and little—turn **blue or blue-black**. The **cytoplasm** of the starch-containing cells being rich in proteins turns **yellow** and may be seen here and there at the edge of the section. Any **strings of gluten** which may be in your preparation also stain **yellow**. Look for them.

Drawings.—Make a careful drawing showing as clearly as you can: (a) the **pericarp** (fruit-coat), (b) the **testa** (seed-coat) with its red and white layers, (c) the **outer endosperm or aleurone layer**, and (d) the **inner endosperm or starchy endosperm**. Put in the cell-contents (**aleurone grains, starch grains**).

The drawing should be about three inches wide. Each of the square-looking cells of the aleurone layer should have its sides about half an inch long and the rest of the drawing should be made in proportion. This layer might be drawn first. The walls of its cells are thick enough to be made with two lines each. The cell-walls of the inner endosperm are so thin that they should be drawn with a single line each. Indicate all the parts which you draw.

Draw a few **starch grains** on a larger scale by themselves. Show their exact shapes and represent some from both the large-size and the small-size groups.

B. THE HISTOLOGY (CELL-STRUCTURE) OF THE ENDOSPERM OF A COFFEE SEED

Preparation of Coffee Seeds for Section Cutting.—Some Coffee seeds were placed in water for 12–24 hours to soften them. Take

one of the seeds, lay it flat on the table and, with a sharp scalpel or pen-knife, cut off one of its ends about one-quarter the way down the seed.

Cutting and Mounting the Sections.—Without wetting your razor or the material, cut some thin transverse sections from the prepared surface, and mount them in water in the usual way.

Examination of the Sections.—Find the best section with the low-power objective, and then study it with the high-power. The cells can readily be seen. Notice that the **cell-walls** are **white** or colourless and have local **nodular thickenings** upon them. Most cell-walls have parallel sides, but here is an exception to the rule. The **cellulose** making up the cell-walls of the Coffee endosperm is not ordinary cellulose such as is present in most cell-walls, e.g. those of cotton fibres, epidermal cells, etc., but is **hemi-cellulose** or **reserve cellulose**, a kind of cellulose which is readily dissolved in weak acids and hydrolised by them into **sugar**. It is stored up in the Coffee seed as a **reserve food material**. Like starch it is a carbohydrate. Its chemical formula is $(C_6H_{10}O_5)_m$. When the seed germinates, an enzyme called **cytase** is produced in the cells by the living protoplasm and this hydrolises the hemi-cellulose into sugar which can be utilised by the embryo in developing into a seedling. As germination proceeds, therefore, the white nodular cell-walls of the endosperm are gradually broken down and disappear.

Owing to the thinness of your section, most of the cells may have been cut open by the razor and part or the whole of their contents may have fallen out. **Unbroken cells have very dense granular contents.** The contents consist of protoplasm (cytoplasm and a nucleus) in which certain reserve food materials are embedded. Most of the **fine granules** appear to be **aleurone grains**.

Among other contents of the cells of the endosperm of the Coffee seed is an alkaloid called **caffeine**. Its chemical formula is $C_8H_{10}N_4O_2$. We cannot observe it microscopically, but it is present to the extent of 0·8–1·7 per cent. in dry Coffee seeds. It is also present in tea leaves, cocoa beans, and kola nuts. Caffeine is a powerful cerebral stimulant, but it also acts somewhat on the heart; it increases the excretory activity of the kidneys or, in other words, it is a strong diuretic. Caffeine is readily soluble in hot water; so, when coffee is made by boiling ground-up coffee endosperm in water, the caffeine passes in solution into the

beverage. A hypodermal injection of caffeine, like drinking very strong coffee, prevents one from going to sleep at the usual time.

Caffeine crystals may be obtained from Coffee endosperm by **microsublimation**. A few ground-up seeds are placed in a small porcelain cup. Then a glass slide is laid across the top of the cup, and the cup is heated from below by a tiny Bunsen by-pass flame. In the course of 5–15 minutes caffeine vapour is driven off from the endosperm and crystallises out in the form of numerous minute slender needles on the under side of the slide. A few slides showing caffeine crystals have been prepared and may be examined with the microscope, if time permits, when the rest of the work in this Chapter has been completed.

Is there any Starch Present in the Endosperm?—Remove the cover-glass of the preparation already made, suck away the water from the section with blotting-paper, and then **add a drop of iodine**. Stir the section in the iodine with a needle, and then add a cover-glass. Examine with the microscope.

If any grains in the preparation have stained **blue** with the iodine, starch is present in the endosperm; if not, starch must be absent. Record the result of the test in your book.

The granular contents of the unbroken cells turn **yellowish**. This we should expect, if **aleurone grains** are present.

Is there any Oil Present in the Endosperm?—Cut another section with your razor. Put it on a new slide in a very **small drop of water**. Add to the drop a drop of **Sudan III**, and then add a cover-glass. Examine with the microscope. If oil is present, it will soon become a **brilliant orange-red**, and the drops so stained will look like oil-drops. Record the result of the test in your book.

Would it be correct to state that, in a Coffee seed, the carbonaceous reserve food materials consist in part of **hemi-cellulose** and in part of **oil**, and the **nitrogenous reserve food material** of **aleurone grains**?—Record your answer.

Drawing.—Make a drawing showing a few cells of the Coffee endosperm with their cell-walls having their characteristic nodular thickenings. Add the cell-contents as best you can. Each cell should be made about 1.5 inches long. As before, indicate with names what has been represented.

CHAPTER IX

THE DATE SEED AND ITS HORNY ENDOSPERM. THE EPI-
DERMIS OF A SCALE-LEAF OF AN ONION. PLASMOLYSIS.

Materials and reagents supplied :

- (1) A whole Date seed ;
- (2) A half Date seed, showing the embryo ;
- (3) A fleshy scale-leaf of a Spanish Onion bulb ;
- (4) A permanently mounted microscopic preparation of a section of Date endosperm, stained with Orange G ;
- (5) A freshly made microscopic preparation of Date endosperm, showing the oil stained with Sudan III ;
- (6) Potassium nitrate (KNO_3), 10 per cent. solution, for plasmolysing living cells ;
- (7) Iodine.

A. THE DATE SEED AND ITS HORNY ENDOSPERM

The Date Palm, *Phoenix dactylifera*, belongs to the great monocotyledonous tropical family of Flowering Plants known as the **Palmae**. It grows in North Africa, Arabia, and Persia. Its trunk is tall and straight without branches, and at its top it bears a crown of 40–80 leaves generally 8–10 feet long amongst which the fruit hangs in bunches. The trunk furnishes timber, the leaves are used for making bags, mats, fans, etc., and the fruit gives food to millions of people. It has many other uses and must be considered as one of the most useful plants in the world. There is a Date Palm in the conservatory at Assiniboine Park. Look for it and for the Banana plant the next time you go there.

1. A Macroscopic Study of the Date Seed.

Examine the **whole Date seed** provided. Notice that it has a longitudinal **furrow** on one side and is **rounded** on the opposite side. The **testa** is a very thin inseparable brown layer which encloses the other parts of the seed and gives to the seed its brown colour. In the middle of the rounded side of the seed find a **small round depression** in the testa. This depression, which is

only about 1 mm. in diameter, marks the position of the embryo in the seed.

Now examine the **half Date seed** provided. To obtain it, a seed was soaked in water for 24 hours to soften it somewhat, and it was then cut in two in the middle by means of a knife which was struck by a hammer. Notice the thin brown **testa** on the exterior of the seed, the **furrow** extending half-way across the seed and therefore resembling the furrow in a grain of wheat, the tiny white **embryo** lying just beneath the testa, and the relatively massive, bluish-grey, horny **endosperm** which completely envelopes the embryo except where this is covered by the testa. The embryo is a mere rod with the bluntly ending **radicle** pointing outwards and a **single conical cotyledon** pointing inwards. The rudiment of the plumule is enclosed by the sheathing cotyledon and cannot be seen. When the embryo is developing into a seedling (see diagram on wall), the end of the cotyledon remains in the endosperm and absorbs nutriment from it.

In having an endosperm, the Date seed resembles the seeds of the Coffee and Castor Oil plants and also the grains of the Gramineae, and it differs from the seeds of the Pea, Bean, Pea-nut, etc., in which there is no endosperm. In that the embryo has only one cotyledon, the Date seed resembles the grains of Wheat, Rye, Oats, Barley, Corn, etc. Palms, like grasses, all belong to the great group of plants known as the **Monocotyledons**.

Make the following drawings : (1) the whole seed showing the furrowed side, (2) the whole seed showing the rounded side with the central depression marking the spot where the testa covers the embryo, and (3) the surface of section of the half seed. (1) and (2) may be drawn twice the natural size and (3) two or three times the natural size. In (3) indicate the **testa**, **furrow**, **embryo**, and **endosperm**.

2. A Microscopic Study of the Endosperm of the Date Seed.

It would be possible for you to cut your own sections of the Date endosperm, after this had been well soaked in water, but the endosperm is so hard that you might seriously blunt your razor edge. Therefore sections have been cut for you by one of the demonstrators. Two sections are to be examined.

Section I.—In this the very thick cell-walls have been stained

with a dye known as **Orange G**, and the section has been permanently mounted in Canada balsam.

Examine the section first with the low-power objective and then with the high-power. You will notice that the **cell-walls are extraordinarily thickened**. Since they have been stained an orange colour, there is no difficulty in distinguishing them. When the cells were very young, their walls were very thin, like those of the cells of the endosperm of the Castor Oil seed ; but, as the seed ripened, layer after layer of **hemi-cellulose** was added to the walls by the protoplasm, until at last the walls attained the extraordinary thickness which you can now perceive. As the walls thickened, each lumen (cell-space) became more and more reduced in size, so that now we find in the section more wall than lumina (cell-spaces). The hemi-cellulose in the endosperm of the Date seed is **reserve cellulose**, and it is **converted into soluble sugar** when the embryo is developing into a Date seedling. The embryo uses up the sugar during its growth.

As the cell-walls thickened, thin places were left in them here and there. These thin places are known as **pits**. A **pit** in the secondary thickening of the cell-wall of one cell is always opposite to a similar pit in the secondary thickening of the cell-wall of the adjacent cell, and a thin **closing membrane** separates the two pits from one another (see diagram on the blackboard). There are **minute perforations through each closing membrane** through which the living protoplasm of one cell communicates with the protoplasm of the adjacent cell. Thus there is what is called **protoplasmic continuity** of the protoplasm in all the cells of the endosperm. The perforations, however, are **too minute to be observed** in your sections.

Make a drawing showing a few cells of the endosperm. Each cell should be about two inches long. Put in the outline of the middle of the wall of each cell first by means of a single line. This line will represent the original thin wall, now called the **middle lamella**. Then add the **secondary thickening** to the wall of each cell, leaving thin places for the **pits**. Indicate the various structures just named and also the **cell lumen** (space) of each cell.

Section II.—In this section the thick cell-walls show their natural whiteness, but the oil in the cell-lumina has been coloured **orange-red** with Sudan III.

From your study of Sections I and II, it is evident that the **carbonaceous reserve food material** in the Date endosperm is

chiefly **hemi-cellulose** stored in the cell-walls, but that this is supplemented by a certain amount of **oil** contained in the cell-lumina. The other cell-contents, namely, cytoplasm, nucleus, aleurone grains, etc., we have not time to study. **Record in your drawing-book** the nature of the carbonaceous reserve food materials.

B. THE EPIDERMAL CELLS OF THE SCALE OF AN ONION

Material.—You have been provided with part of one of the fleshy scale-leaves of a Spanish Onion bulb. The **smell of garlic** is due to an oil, **oil of garlic**, contained in the tissues. The oil is made up of sulphur compounds, chiefly allyl-sulphide, $C_6H_{10}S$, but also triallylsulphide, $C_6H_{10}S_3$, which has the characteristic odour of garlic.

To make a Preparation of Epidermis.—Put a drop of water in the middle of a slide. Take the piece of onion scale and find its **inner** or **concave side** (the one that faced the centre of the bulb). Now with the help of your fingers and a pen-knife or scalpel **peel off a piece of the epidermis**. It comes away from the sub-epidermal tissues very readily in the form of a thin sheet. Handle the epidermis carefully, as its cells are to be studied whilst still living. Lay the piece of epidermis on the drop of water on the slide. Put **another drop** of water above it and then add a cover-glass. If the piece of epidermis taken from the scale is too large, it can be cut down with a pair of scissors or, when it is lying on the slide, with a scalpel or knife. A fairly large piece about 1 cm. square or one-half inch square would not be too large.

Observations with the Low-power Objective.—Note that the epidermis consists of a **single layer of cells** and that the epidermal cells are all **elongated in the same direction** (this direction corresponds to the long axis of the scale). There are **no intercellular spaces** between the cells: verify this by careful examination of the walls of the cells where three cells meet. The **cell-walls** are **very thin**. Compare the thickness of a wall with the width of a cell. The **cell-contents** are **colourless**. There is a fairly conspicuous **nucleus** in each cell.

Observations with the High-power Objective.—After making out the points described above, use the high-power objective for studying some individual cells.

A single cell is bounded by a thin dead **cell-wall** made of

cellulose. Lining the cell-wall and pressed closely against the cell-wall is a thin **layer of cytoplasm** in which minute round granules are scattered.

The **nucleus** is large and should be readily found. It is embedded in the cytoplasmic layer already mentioned. If the nucleus is just below the upper wall of a cell or just above the lower wall, it has a **rounded** outline ; but, if it is on a side-wall, it appears to be **plano-convex**. Is the nucleus shaped like a plano-convex lens ? If the nucleus is well focussed and properly lighted, you will find in it a **nucleolus** or perhaps two or three **nucleoli**. These are small round bodies. You may also be able to perceive that the nucleus is finely **punctate**, i.e. has a dotted appearance.

Focus a cell-wall carefully and try to observe the **thin cytoplasmic layer** on each side of it. Each layer is not much thicker than the cell-wall itself and contains minute shining particles scattered in it. These particles may sometimes be seen moving along in a direction parallel to the cell-wall. The cytoplasmic layer appressed to the cell-wall is known technically as the **primordial utricle** (utricle means a sack). The primordial utricle encloses a very large space known as a **vacuole**, and the vacuole is filled with a watery fluid called **cell-sap**. If you focus half-way down a cell, you will see the cell in **optical section**. In this section you will see just the lateral walls of the cell lined with cytoplasm and the huge perfectly clear vacuole filled with colourless cell-sap occupying all the centre of the cell.

In a favourable preparation you may observe here and there in a cell one or more **protoplasmic bridles** or strings of cytoplasm stretching through the large central vacuole from one side of the cell to the other. The particles in these bridles may be quite still, or they may move fairly rapidly along the bridles from one place to another, in which case we have an instance of what is known as **protoplasmic streaming**.

Make a drawing of two or three cells. Each cell should be drawn three or four inches long. Show the **cell-wall** (drawn with two close parallel lines), the **absence of intercellular spaces** (indicated by the way the cell-walls fit together), the **nucleus** with its **nucleolus** or **nucleoli**, the **cytoplasm** forming a thin layer (**the primordial utricle**) around the cell-wall and surrounding the nucleus, the large central clear space or **vacuole** filled with **cell-sap**, and one or more **protoplasmic bridles**, if you have observed them.

C. THE PHENOMENON OF PLASMOLYSIS

1. **Theoretical Explanation.**—In living cells, such as the epidermal cells of the onion, the cell-sap in the vacuole presses the primordial utricle against the cell-wall with a pressure equal to several atmospheres, and this pressure is counterbalanced by the tension in the distended cell-wall (compare the pressure of the air in a blown-up football). A cell hydrostatically distended in the manner just described is said to be **turgid** or to exhibit **turgidity**. Like a blown-up football, it is a rigid structure.

A cellulose cell-wall is permeable to both water and salts, etc. But a **layer of protoplasm** is a **semi-permeable membrane**. Normally the primordial utricle does not allow substances dissolved in the cell-sap, e.g. sugar, salts, etc., to escape from the cells when the cells are surrounded by water, but it is permeable to water. Hence, by **osmosis**, water passes through a primordial utricle into a living cell until the cell becomes distended, the limit of distension being determined by the resistance of the cell-wall. Thus the pressure of the cell-sap in a living cell is **osmotic pressure**. This pressure exerted on the cell-wall by the cell-sap is often equal to 5–20 atmospheres. The inward pressure of the cellulose wall on the cell-sap must therefore also be often equal to 5–20 atmospheres.

It is possible to cause the protoplasmic contents of a cell to contract away from the cell-wall or, in other words, it is possible to **plasmolyse the cell** by osmosis. This may be accomplished by **putting the cell in a salt solution which osmotically is stronger than the solution of salts, sugar, etc., present in the vacuole**. Water then passes by osmosis from the vacuole through the semi-permeable protoplasmic layer (primordial utricle) into the salt solution bathing the cell. In consequence of the loss of water from the cell, the cell-sap has to take up a smaller space. On this account the protoplasm contracts away from the cell-wall. As the vacuole gets smaller and smaller, the salts, etc., in the cell-sap become more and more concentrated. When this concentration attains that of the strong salt solution in which the cell has been bathed, equilibrium is established. Then osmosis ceases and the protoplasm contracts no farther.

2. **Experimental Plasmolysis.**—With the help of blotting-paper run some 10 per cent. potassium nitrate, KNO_3 , under the cover-glass of your preparation and, immediately thereafter, with the

low-power first and then the high-power, watch what happens to the cell-contents.

You will observe, if the cells are living, that the **primordial utricle contracts away from the cell-walls** and gradually forms a series of balls or rounded masses of protoplasm. The salt solution has penetrated through the cellulose walls into the cells and is now in contact with the exterior of the protoplasm. Soon equilibrium becomes established and there is no further contraction of the protoplasm.

What has happened? We have put the cells in a solution, 10 per cent. KNO_3 , which has a very high osmotic pressure—about 30 atmospheres—whereas the osmotic pressure of the cell-sap in the vacuoles due to the pressure there of salts, sugar, etc., is relatively low, say 5–10 atmospheres. Hence osmosis has taken place. Water diffused out of the vacuoles through the semi-permeable primordial utricle into the exterior potassium nitrate solution until the salts, etc., in the cell-sap of the vacuoles attained a concentration equal to that of the potassium nitrate solution in which the cells were placed. Then equilibrium was established and osmosis ceased.

Make a drawing of a few plasmolysed cells, and in it indicate where the potassium nitrate solution is present.

3. De-plasmolysis.—Replace the potassium nitrate solution with water by running this under the cover-glass with the help of blotting-paper. **Immediately after** you have done this, find some plasmolysed cells with the low-power and then study them with the high-power. You should be able to observe that the **protoplasm in the cells expands again** so that each cell comes to look like it did before it was plasmolysed. You should be able to **watch the primordial utricles going back to their old positions**.

What has happened? The potassium nitrate having been replaced by water, **osmosis** has taken place. **Water has passed into the contracted vacuoles**, so that the contents of the vacuoles have become larger and larger, until finally the cell-sap has again pressed the primordial utricle against the cell-wall. The cell-wall has again become distended, so that its tension just balances the osmotic pressure of the cell-sap.

4. Application of Iodine.—Make a new preparation of a piece of epidermis. Mount in water and then run a drop of iodine under the cover-glass. Suck it through with blotting-paper.

Note that the cell-walls and the protoplasmic contents of the

cells all stain yellow. The nucleus becomes deep yellow. The cytoplasm and nucleus are both made up chiefly of proteins, and proteins turn yellow with iodine. The iodine solution is **poisonous** and it **killed the cytoplasm and nucleus** as soon as it came into contact with them.

5. Can Dead Cells be Plasmolysed?—Remove the slide from the stage. Remove the cover-glass from the preparation. Suck away the superfluous iodine with blotting-paper. Then add a drop of 10 per cent. potassium nitrate. Stir the epidermis in the salt solution and then add a cover-glass.

Are any of the cells plasmolysed? If not, why not? May we conclude that dead cells cannot be plasmolysed? May plasmolysis be used as a test to decide whether a plant cell is living or dead? Record your answers in your book.

6. How would you define Plasmolysis? Record your answer in your book.

CHAPTER X

A FURTHER STUDY OF PLANT CELLS WITH SPECIAL REFERENCE TO THEIR RED, BLUE, AND YELLOW PIGMENTS

Materials and reagents supplied :

- (1) A piece of the red crown-tuber (beet-root) of a Garden Beet;
- (2) A Violet flower or some blue ray florets of a Cineraria ;
- (3) A piece of the orange-yellow crown-tuber of a Carrot ;
- (4) Some florets of a yellow Chrysanthemum ;
- (5) Potassium nitrate, 10 per cent. solution, to be used for plasmolysing living cells ;
- (6) Iodine (dissolved in an alcoholic solution of potassium iodide), to be used for killing the protoplasm of plasmolysed cells and for staining the protoplasm yellow ;
- (7) Hydrochloric acid, dilute, to be used for killing the protoplasm of plasmolysed cells and for turning violet anthocyanin red.

A. BEET-ROOT

The Beet (*Beta vulgaris* or *B. maritima*) is closely allied to Spinach (*Spinacia oleracea*) and to the common weed called Pigweed or Lamb's Quarters (*Chenopodium album*), and all

these three plants are included in the natural family **Chenopodiaceae**. Among the varieties of the Beet species are: (1) the **Garden Beet**, eaten as a vegetable, (2) the **Sugar Beet**, a most important source of cane-sugar, and (3) the **Mangold-wurzel**, of high value in feeding cattle.

Beet is a biennial plant. In its first year it grows from seed, and produces a crown of leaves but no flowers or seeds, and its primary root and its stem bearing the crown of leaves become greatly swollen, so that together they form a **crown-tuber**—the beet-root of commerce and of field husbandry. The crown-tuber is a **magazine in which reserve food materials are stored up**. The chief food material so stored is cane-sugar (sucrose, saccharose), $C_{12}H_{22}O_{11}$, i.e. the same kind of sugar as that stored up in the cells of the Sugar-Cane (*Saccharum officinarum*). Most of the cells in the crown-tuber of the Beet are thin-walled and isodiametric, and the **sugar** in each cell is **dissolved in the cell-sap** of a very large central vacuole. In its second year, the Beet plant produces numerous slender branched stems which bear flowers, fruits, and seeds. The development of these structures takes place at the expense of the sugar, etc., which was stored up in the crown-tuber during the previous year. The crown-tuber gradually becomes exhausted of its reserve food materials as the fruits and seeds are developed; and, as the seeds ripen, the plant dies.

To make a Microscopic Preparation of Beet-root.—A piece of the crown-tuber of a red Beet has been supplied to you. With a pen-knife or scalpel cut from it a block of tissue which you can conveniently hold for section-cutting. Then, with your razor, cut from the block a transverse or a longitudinal section. Before beginning to cut the section, lubricate your razor and the material with water. Mount the section in water in the usual way and examine it with the microscope. **Wipe your razor dry**, lest its edge becomes corroded by the beet-root sap.

Observations with the Microscope.—Note that the root is made up chiefly of rectangular cells and that many of the **intercellular spaces** appear as **dark streaks** owing to their being filled with **air**.

The **cell-walls** are very thin and the **cell-lumina** (cell-spaces) large. In each cell there is a thin layer of cytoplasm, the **primordial utricle**, pressed against the cell-wall by the **cell-sap** in the very large central vacuole. Here and there, embedded in the cytoplasm, you may be able to observe a **nucleus**.

The **large central vacuole** in each cell contains **watery cell-sap** in which various substances are dissolved. One of these substances is **anthocyanin**, a red colouring matter. To this substance the **red colour** of many of the cells is due. Another substance dissolved in the cell-sap, **cane-sugar**, cannot be seen as it is colourless, but it is of high commercial importance. In the Sugar-beet, grown on farms for the extraction of sugar, the sugar in the cell-sap varies from 12 to 19 per cent.

Note the red colour of the vacuole of many of the cells and the degree of redness.

Make a sketch showing one or two cells. Write above the drawing : **Cells of Beet-root before Plasmolysis.** The cells should be at least an inch wide. Indicate the **cell-wall**, the **primordial utricle**, the **vacuole containing red cell-sap**, and a **nucleus**, if you have seen one. Do not spend too much time on the drawing.

Plasmolysis.—Take your slide off the stage of the microscope and place it on the table. Remove the cover-glass, suck away from the section any water left around it, and then add a large drop of **10 per cent. potassium nitrate**. Stir the section in the drop for 0·5–1 minute so that the salt solution can act upon all the cells, and then add a clean cover-glass. Remove any superfluous salt solution around the edge of the cover-glass, and then observe the preparation with the microscope.

It should be at once apparent that many of the cells have become plasmolysed. The cytoplasm in each plasmolysed cell will be seen to have contracted away from the cell-wall, so that it now forms a more or less rounded ball. **Note the degree of redness in the cell-sap** in the plasmolysed cells. Is it not greater than that seen in the section before plasmolysis took place ?

The **contraction of the cytoplasm** away from the cell-wall, as explained in Chapter IX, was **caused by osmosis** ; the osmotic pressure of the 10 per cent. potassium nitrate solution in which the cells were bathed was greater than the osmotic pressure of the sugar and salts, etc., which were present in the vacuoles. Hence, by osmosis, water passed out of the vacuoles through the semi-permeable protoplasmic membrane (primordial utricle) into the potassium nitrate solution until the osmotic pressure inside and outside each plasmolysed cell became equalised. As the water passed out of a vacuole, the cell-sap became more and more reduced in volume and the cytoplasmic layer in consequence was drawn away from the cell-wall. The **cane-sugar** and the

anthocyanin pigment were not able to penetrate through the protoplasm, since this substance was not permeable for them. Hence they remained in the vacuole ; and, as the vacuole became smaller and smaller, they became more and more concentrated. The concentration of the red pigment is indicated to the eye by the deepened colour of the vacuoles.

Make a semi-diagrammatic drawing showing five or six cells with plasmolysed cell-contents. Write above the drawing: **Cells of Beet-root after Plasmolysis.** Indicate the cell-wall, primordial utricle, and vacuole containing concentrated anthocyanin pigment. You might shade the vacuole lightly in this and your previous drawing sufficiently to indicate the relative depth of colour.

To kill the Plasmolysed Cells with Iodine.—Remove the slide from the stage of microscope and put it on the table. **Remove the cover-glass**, and with blotting-paper suck away any of the salt solution left around it. Then **add a drop of iodine**, stir the section in the iodine for a moment or two so that the iodine has a chance to penetrate into it, add a cover-glass, and observe the preparation with the microscope.

You should be able to observe that the **contracted cytoplasm** in the plasmolysed cells soon **takes on a yellow colour**. The iodine and the alcohol in which it is dissolved kill the protoplasm and alter its physical properties. As soon as the cytoplasmic sack is dead, it is **no longer a semi-permeable membrane** which prevents sugar, salts, colouring matters, etc., from passing through it, but it is a **permeable membrane**. Hence, as the cells die, the sugar, salts, pigment, etc., begin to escape from the vacuoles. **Observe the disappearance of the anthocyanin** from the vacuoles. You will find that the red colour of the cell-sap disappears as the anthocyanin diffuses away and that the vacuoles soon become **colourless**. At the same time, the contracted protoplasm loses its perfectly rounded outline and this **becomes waved or irregular**. When plenty of iodine has penetrated into the cells, you should be able to perceive the **nucleus**, now very yellow, lying in the less yellow cytoplasm.

B. A PETAL OF A VIOLET, OR A BLUE RAY FLORET OF A CINERARIA, AND ITS ANTHOCYANIN

Material.—You have been provided with a petal of a Violet (a species of *Viola*) or, if that is not available, a blue ray floret

of a *Cineraria*. The violet colour is due to the presence of **anthocyanin**.

The **anthocyanins** are a complex group of colouring matters which, in the main, give to **red** and **blue** flowers, etc., their peculiar colours. We have already met with a red anthocyanin in the Garden Beet. The **red colour** of Roses, Poppies, and Geranium flowers, as well as of the roots of Beets and Radishes, is due to anthocyanins. Likewise the **blue colour** of Violet, Larkspur, and *Cineraria* flowers, as also of the fruits of Grape-vines and Blueberry bushes, is **due to anthocyanins**.

It is to the development of a red anthocyanin pigment that we owe the **autumnal redness** of many leaves, including that of our national emblem, the **Maple Leaf**.

Like litmus, some anthocyanins are **blue** or **violet** in an alkaline solution and **red** or **reddish** in an acid solution. **Is this so for the anthocyanin of the Violet?** This may be tested both macrochemically and microchemically.

Macrochemical Test.—Cut off the **lower** narrower half of the petal provided. Put a small drop of **dilute hydrochloric acid** on the middle of the slide. Be careful with this acid; do not get spots of it on the table. Then lay the lower half-petal on it with the help of a needle, so that it lies flat on the slide. **Do not** add a cover-glass. Place the slide over some white paper, e.g. a strip of blotting-paper, and watch what happens in the course of a minute or two. **Record in your book** whether or not the anthocyanin in the petal, when acted upon by the acid, turns from blue to red. **Wash** your needle in water.

To make a Microscopic Preparation of a Violet Petal.—Lay the **upper** broader half of the petal on a clean glass slide and, with a pen-knife or scalpel, cut it across twice so as to obtain from it a narrow band of tissue about 1–2 mm. wide. Scissors may be used instead of a knife. Put a drop of water on the slide, flatten the band of tissue out in it with a needle, add another drop of water and then a cover-glass. Observe the preparation with the microscope.

Observe with the low-power objective that the **petal owes its violet colour to the violet cell-sap in the epidermal cells** and that the black lines and streaks are due to **air** imprisoned in intercellular spaces. Find a place in the preparation which is fairly free from air-spaces and observe some of the cells with the high-power objective. You should be able to see the **cell-walls** which

are curiously waved. Notice that the **violet colour** of each cell is due to the anthocyanin pigment dissolved in the cell-sap filling the large central vacuole. The **colourless cells** at the edges of the section have lost their pigment owing to injury. The structure of the epidermal cells is similar to that of the cells of a beet-root.

Plasmolysis.—Run plenty of **10 per cent. potassium nitrate solution** under the cover-glass so as to replace the water. Observe with the microscope that the cells, particularly those near the cut edges of the preparation, soon become strongly plasmolysed. The cytoplasm in many of the cells may become contracted into almost or quite spherical balls under the influence of the salt solution. Notice the deepening of the violet colour in the vacuoles, which indicates the inability of the anthocyanin to pass through the cytoplasmic membrane and its increasing concentration.

Make a semi-diagrammatic drawing showing two or three plasmolysed cells. Use your own judgment about the scale.

Microchemical Test of the Influence of an Acid on the Anthocyanin of the Violet.—Remove your preparation from the stage of the microscope and lay it on the table. Suck a little of the salt preparation away from beneath the cover-glass, and then run under the cover-glass a **drop of dilute hydrochloric acid**. Immediately thereafter observe with the microscope the effect of the acid upon the plasmolysed cells. (Be careful not to get any acid on the microscope. If any acid should get on to an objective, the objective should be removed, dipped in water, and wiped dry.)

As soon as the acid comes into contact with a plasmolysed cell, the **anthocyanin** held in the vacuole **turns from violet to red**. After a short time, the cytoplasm is killed by the acid and the **reddened anthocyanin** then diffuses out of the vacuole into the surrounding medium. Observe for yourself the two changes just described. Record them in your book.

C. THE CARROT AND ITS CAROTIN

The Carrot, *Daucus Carota*, like the Beet, develops a swollen **crown-tuber**. The crown-tuber has a characteristic **orange-red colour**. This colour is not due to an anthocyanin, but to a pigment known as **carotin**; and the carotin is not dissolved in cell-sap but **is present in tiny specialised protoplasmic bodies** known as **chromoplasts**. The protoplasm in the cells of the Carrot root, therefore, is divisible into **cytoplasm, nucleus, and**

red chromoplasts. The chromoplasts, like the nucleus, are embedded in the cytoplasm.

To make a Preparation of a Carrot Root.—A piece of the crown-tuber of a Carrot is provided. A section is to be cut from its **reddest part**, namely the outer **cortex**. With a pen-knife or scalpel, shave off the dirty layer on the outside of the piece of carrot. You will thus expose a tangential surface of the outer cortex. From this surface cut a **surface section** (i.e. a section parallel to the surface). Mount the section in water, add a cover-glass, and examine with the microscope. Use your own judgment in cutting down the piece of carrot into a block suitable to hold in the left hand, and also in the matter of lubricating the razor and material with water before the section is cut.

You will readily observe that the cells have thin walls and that they contain **carmine and orange-red colour-bodies which possess a crystalline form**. The large central **vacuoles** of the cells are **colourless**, so that it is evident that the orange-red colour of the Carrot root is due to the **solid orange-red colour-bodies or chromoplasts**. The colouring matter of the chromoplasts, **carotin**, has the chemical formula $C_{40}H_{56}$ and, as it crystallises out, determines the shape of the chromoplasts in which it is formed. The most common forms of the chromoplasts are rectangular plates or rhombs. The rhombs are often acicularly elongated. The prisms are of different lengths, often broadened out at one end so as to be fan-shaped. Sometimes one or more very tiny starch grains may be seen adhering to a chromoplast.

The orange-red chromoplasts are **embedded in the cytoplasmic layer** pressed against the cell-wall. In general, the structure of a cell of the Carrot root resembles that of the cells of the Beet-root and of the epidermal cells of the Violet, but we have not time to study its details any further.

Make a drawing of one or two cells of the Carrot root. Each cell should be 1–1.5 inches long. Indicate what the drawing is. Show the **cell-walls** and a number of **chromoplasts**. The other details may be omitted.

D. A YELLOW CHRYSANTHEMUM AND ITS PIGMENT

Yellow Chrysanthemum flowers owe their colour not to anthocyanin but to a yellow pigment which is probably a form of **carotin**, and this, like the carotin in a Carrot root, is not

dissolved in cell-sap, but is present in tiny specialised masses of protoplasm known as chromoplasts.

To make a Preparation of the Petals of a Yellow Chrysanthemum.—In the same manner as that described for the Violet petal, cut away from the united petals of the yellow chrysanthemum floret a narrow transverse band about 1–2 mm. wide. Mount in water under a cover-glass and observe with the microscope.

Observations with the Microscope.—Find the epidermal cells and observe the numerous **tiny yellow chromoplasts** embedded in the cytoplasm of each cell. The chromoplasts are rounded or in the form of short bluntly-ending rods. The yellow pigment present in the chromoplasts is not crystallised out but is in an amorphous form. In this it is different from the carotin of the Carrot.

Proof that the chromoplasts are embedded in the cytoplasm may be obtained by plasmolysing the cells with 10 per cent. potassium nitrate. **Plasmolyse the cells** and observe the presence of the yellow chromoplasts in the contracted cytoplasmic sac.

Make a sketch of some epidermal cells showing yellow chromoplasts embedded in the cytoplasm. Indicate what your drawing is meant to represent.

CHAPTER XI

THE LEAF OF A MOSS PLANT AND ITS CHLOROPLASTS. STORAGE STARCH IN A POTATO TUBER

Materials and reagents supplied :

- (1) A leaf of a Moss plant ;
- (2) A piece of a Potato tuber, including rind and pith ;
- (3) Potassium nitrate, 10 per cent. solution, to be used for plasmolysing living cells ;
- (4) Alcohol, 95 per cent., to be used for killing plasmolysed cells and for removing the chlorophyll from chloroplasts ;
- (5) Iodine (dissolved in an alcoholic solution of potassium iodide), to be used for staining blue-black the starch grains contained in chloroplasts.

A. THE LEAF OF A MOSS PLANT AND ITS CHLOROPLASTS

Macroscopic Examination.—A living leaf of a Moss plant (*Rhodobryon roseum* or some other species) has been provided.

Mount it on a slide in a drop of water under a cover-glass, and examine it with the naked eye. Notice its uniform green **colour**, its **shape** (broad at the base, still broader toward the middle and pointed at the apex), and its thin **mid-rib** or **median vein** running up its central axis to the apex.

Make a sketch of the leaf showing its exact shape and its mid-rib. Make the leaf about one inch long. You should be able to make an accurate drawing in less than two minutes.

Microscopic Examination.—Examine the leaf with the **low-power objective**. Note the bundle of **narrow elongated cells** making up the mid-rib. These cells served to conduct substances in solution from or to the stem on which the leaf was situated.

Observe that on each side of the mid-rib the **lamina is only one layer of cells in thickness**. The leaves of Flowering Plants are many layers thick and have an upper and lower epidermis. In comparison with them, therefore, a Moss leaf has an exceedingly **simple structure**.

The whole leaf was formed by division of an **apical cell**. Look for this apical cell at the leaf apex (low-power first, then high-power).

Notice first with the low-power objective and then with the high that along the **leaf margin**, toward the apex of the leaf, the apical end of each cell is extended forward so as to form a **tooth**. The margin of the leaf may be described as **dentate** (toothed). Is or is not the cell-wall at the end of each tooth thickened?

Observe that the **cells of the lamina on each side of the mid-rib** fit tightly together, so that here, as in the epidermis of an Onion scale, there are **no intercellular spaces**. The cell-walls are colourless and therefore are white in appearance. The cells are polygonal (many-sided) in shape. **How many sides has each cell as a rule? How many neighbouring cells has each cell surrounding it?** Notice that each cell is elongated in the same direction as the long axis of the leaf.

The most striking objects in the cells of the lamina are the **chloroplasts** or **chlorophyll corpuscles**. There are a number of them in each cell. They are all embedded in the primordial utricle (cytoplasmic sac) which, as in other cells you have studied, lies just within the cell-wall. Evidently, the **green colour of a Moss leaf** is entirely due to the presence of the numerous chloroplasts contained within its cells.

With the high-power objective and with the optimum light

(strongest blaze of light up the tube from the mirror, then light cut down with the iris diaphragm) **focus some chloroplasts carefully**. You will see most of them in **face view** as they lie in the cytoplasm just beneath the upper horizontal cell-wall. Thus seen, they appear with a **round or oval outline**. If, however, you can find any chloroplasts on a lateral wall (try the cells at the leaf-margin) you will find that they have a **flattened** appearance. Since the chloroplasts are rounded in face view and flattened in side view, their form must be **discoid**.

When a Moss leaf is developing, the chloroplasts increase in numbers by simple division : a chloroplast may elongate, become constricted in the middle like a dumb-bell, and then break in the middle so as to form two chloroplasts. You should be able to find some **chloroplasts in the dumb-bell stage**. Look for them.

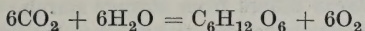
Each of the green cells consists of a **cell-wall** made of cellulose, a thin layer of **cytoplasm** (the **primordial utricle**), in which a **nucleus** and the **chloroplasts** are embedded, and a large central **vacuole** filled with clear **cell-sap**. By carefully focussing a single cell with the high-power objective, you may or you may not be able to perceive something of the thin colourless primordial utricle just underneath the upper horizontal cell-wall ; but you are not likely to see the nucleus, as it is hidden by the green chloroplasts.

Starch Grains in the Chloroplasts.—Carefully focus some of the chloroplasts lying just beneath an upper cell-wall and search in them for tiny elongated **starch grains**. There is often one but there may be two or three starch grains in each chloroplast, and they appear as tiny **whitish glistening rods**.

These starch grains are the first **visible** product of the physiological process known as **photosynthesis** (carbon assimilation), a process which can be carried out **only by chloroplasts** and **only in the light**. When exposed to sunlight, the chloroplasts manufacture sugar from the simple raw materials **carbon dioxide** and **water**. The sugar so formed, being soluble, diffuses out of the chloroplasts into the cell-sap and from one cell to another, and from the leaf to the stem, etc. ; but, when there is a **surplus of sugar**, some of it is **got rid of temporarily** by being converted into **insoluble starch grains**. It is starch so formed that you can now perceive in the chloroplasts.

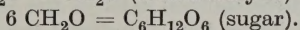
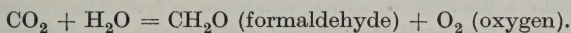
The formation of sugar from carbon dioxide by the chloroplasts during photosynthesis may be represented by the following

chemical equation, which may be called the **general equation for photosynthesis** :—

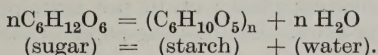


carbon dioxide + water = sugar + oxygen (a waste product).

It is probable, however, that photosynthesis takes place in a stepwise manner, **formaldehyde** (a very simple carbohydrate) being formed first, and the formaldehyde being then polymerised into sugar (a much more complex carbohydrate). This stepwise synthesis of sugar can be represented by two chemical equations, thus :—



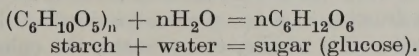
Remember that **sugar** is the **chief synthetic product of photosynthesis**. The process by which surplus sugar is converted into solid starch grains may be represented by another equation :—



Thus starch is formed by the union of many molecules of sugar (glucose) with the elimination of one molecule of water from each.

All the **synthetic work** of building up complex sugar molecules from carbon dioxide and water is accomplished only by the aid of **energy** derived from the light of the sun. Hence the term **photosynthesis**. The **light** (chiefly red-orange rays) is absorbed by the green colouring matter **chlorophyll**, and the **light energy** so obtained is somehow converted into **chemical energy** which is made use of by the chloroplast and ultimately stored up in the sugar molecules. In the dark the chloroplasts cannot carry on photosynthesis as the energy (light) absolutely required for the process is not available.

The **starch grains in chloroplasts are only in existence temporarily**. If a Moss plant is kept in the dark for some time, the starch in the chloroplasts is slowly converted into sugar by the action of diastase and therefore completely **disappears** from view. The sugar formed from the starch grains diffuses into the vacuoles, etc. The following equation represents the conversion of starch into sugar :—



The starch is said to be hydrolised into sugar under the action of diastase.

Chloroplast starch differs from storage starch, which you have studied in the seeds of the Pea, the grain of Wheat, and the tuber of a Potato, in (1) the grains being relatively **minute**, and in (2) the **mode of origin**. Chloroplast starch is manufactured directly from CO_2 and H_2O , whereas storage starch is formed by leucoplasts from sugar which was manufactured from CO_2 and H_2O by the chloroplasts in the leaves and then sent to the leucoplasts (in seed, potato tubers, rhizomes, etc.) *via* the vascular bundles (conducting strands of cells).

Make a drawing in which you show a few cells of the lamina including two or three toothed cells at the leaf-margin. Make each cell about 1.5 inches long. Indicate the **cell-walls**, **absence of intercellular spaces**, and the **chloroplasts** with any **starch grains** they may contain.

Plasmolysis.—To observe the primordial utricle, plasmolyse the cells by running plenty of 10 per cent. potassium nitrate under the cover-glass. Use blotting-paper in the operation.

With the low-power objective, find some cells becoming plasmolysed and then, with the high-power, watch the **gradual contraction of the primordial utricle**. The first cells to become plasmolysed are those near the base of the leaf, as there the salt solution can diffuse most readily through the cell-walls owing to the cut surface. As a rule the primordial utricle retires from the **ends** of the cells first. In each plasmolysed cell note how thin the primordial utricle is and that the chloroplasts are embedded in it.

Certain cells do not become plasmolysed. Look for these unplasmolysed cells wherever the leaf has been injured, e.g. along the leaf base. These unplasmolysed cells are all **dead**. Their primordial utricle, having lost its vitality, is **no longer a semi-permeable membrane**, but allows the potassium nitrate solution to pass through it into the vacuole without any resistance. Hence osmosis cannot take place and plasmolysis is impossible. In the plasmolysed cells the salt solution has passed through the cell-walls and now bathes the outside of the contracted primordial utricles, but it cannot get through the utricles owing to their being alive and, therefore, still semi-permeable.

Make a drawing of a few plasmolysed cells. Indicate the contracted **primordial utricle**, the **vacuole**, the **chloroplasts** and, by

means of arrows, to what part of each cell the potassium nitrate solution has penetrated. Make the drawing on the same scale as the previous one.

To Kill the Plasmolysed Cells and to Remove the Chlorophyll from the Chloroplasts by Means of Alcohol.—Remove the cover-glass from the Moss leaf which has been plasmolysed, and suck away the potassium nitrate from around the leaf with the help of blotting-paper. Then add to the leaf a drop of 95 per cent. alcohol, lay on a clean cover-glass, and then examine the preparation with the microscope.

The alcohol soon penetrates through the cell-walls, reaches the protoplasm and kills it. The edges of the contracted primordial utricles appear less sharp and less rounded after the utricles have died.

Chlorophyll is Soluble in Alcohol.—As soon as the 95 per cent. alcohol comes into contact with the chloroplasts, their green colouring matter, the **chlorophyll**, begins to diffuse out of them into the cell-sap inside the vacuoles and through the contracted primordial utricles into the spaces between the utricles and the cell-walls. Soon the **cells appear to be uniformly green** owing to this process of diffusion. Note that the chloroplasts become paler and paler, and that the dead primordial utricles still remain contracted.

In broken cells, e.g. those at the base of the leaf, the chlorophyll can diffuse out of the cells without any hindrance from the cell-walls. Hence in these cells the **chloroplasts soon become quite colourless**. Find some of these decolourised chloroplasts.

If the leaf were to be put into alcohol for some time, it would gradually become entirely decolourised. It would then appear white instead of green. The chloroplasts would be left behind, but their chlorophyll would have diffused away.

To Colour the Starch Grains Formed in the Chloroplasts Blue-black with the Help of Iodine.—Take a fresh Moss leaf, lay it on a slide, add to it a drop of iodine, and then cover it with a cover-glass. Observe with the microscope.

Now **look for the starch grains** in the chloroplasts. Note their colour, their shape, and their number in each chloroplast.

The **cytoplasm** and **nucleus** are coloured yellow by the iodine and are therefore more easily seen than they were in the living unplasmolysed cells. In living cells, the **nuclei** are hidden by the green chloroplasts; but, now that they have been stained yellow,

you should be able to find them. There is one in each cell, and each nucleus contains a **nucleolus**.

To Determine whether or not Cells Killed by Iodine can be Plasmolysed.—Take the preparation just made, lift the cover-glass, remove the iodine with blotting-paper, add a drop of 10 per cent. potassium nitrate solution to the leaf, and then cover the preparation with a clean cover-glass. Do the cells become plasmolysed or not? If not, why not?

B. STORAGE STARCH IN A POTATO TUBER

The White Potato or Irish Potato, *Solanum tuberosum*, is closely related to the Tomato plant (*S. Lycopersicum*), the Egg-fruit plant (*S. Melongena*), the Deadly Nightshade (*Atropa belladonna*), the Jimson-weed (*Datura Stramonium*), the Tobacco plant (*Nicotiana Tabacum*), and to several plants grown for the beauty of their flowers, e.g. *Petunia*, *Salpiglossis*, and *Schizanthus*. All these plants, including the Potato, belong to the great natural family of Dicotyledons known as the **Solanaceae**.

Next after the cereals or grains the Potato is the most valuable of all plants used for human food; and, like Corn (Maize), it was first given to the world by America. It is often called the Irish Potato, but it was not known in Ireland until it was carried there from Virginia, U.S.A., some 350 years ago. It grows wild in Peru, Chili, and other parts of South America, and was carried thence to North America, it is thought, by the Spaniards, for it is not known that any of the Indians grew it before the white men came. It was imported into Europe between 1580 and 1585, first by the Spaniards and afterwards by the English at the time of Raleigh's voyages to Virginia.

About three-fourths of the weight of a full-grown potato is water. The other fourth consists chiefly of starch together with a smaller amount of proteins. The potato also contains a little of a poisonous substance called **solanine**, an alkaloid to which has been attributed the chemical formula $C_{52}H_{93}NO_{18} + 4\frac{1}{2}H_2O$. This poisonous substance is removed by cooking. It occurs especially in the inner layers of the rind and in the neighbourhood of the axillary buds (eyes). It is present also in considerable amount in young shoots produced from potatoes in the spring. Potatoes which have been exposed to the light and have turned green are said to contain much solanine and to be unfit for food.

The turning green is due to the leucoplasts developing chlorophyll and thus changing themselves into chloroplasts.

To Obtain a Section of a Potato Tuber.—Take a piece of potato tuber provided, with a pen-knife cut from it a block of tissue, including a piece of the rind, that you can hold readily in your left hand; and then cut from the block with your razor one or two small thin sections. Lubricate your razor with water. The sections should include one which passes through the rind (outer protective cork layer) and one which has been made through the tissues (pith) nearer the centre of the tuber. Mount in water.

Microscopic Observations.—With the low-power objective first and then with the high, observe that the potato tuber is made up chiefly of **parenchymatous cells**, i.e. of cells which are more or less isodiametric (with equal diameters in all directions), thin-walled, and living. Here and there you may see some air still left in some of the intercellular spaces.

Examine the starch grains lying in the cytoplasm of the cells. Count their number in a single unbroken cell. The grains are just like the isolated potato starch grains you examined earlier in the session. They are mostly simple and conchoidal (like a sea-shell) or ellipsoidal. They vary from about 0.005 to 0.125 mm. in length. Their **hilum** is circular and at the smaller end of the grain, and the **lamellae** are concentric and eccentric.

Leucoplasts.—Each of the starch grains in a potato tuber has been formed by a **leucoplast** or colourless **starch-former**—a specialised living mass of protoplasm very like a chloroplast but without its green colouring matter, chlorophyll. Sometimes, in an uninjured cell, one can see something of the leucoplast attached to and surrounding a very small starch grain, but the leucoplasts surrounding the large starch grains cannot be seen in the preparation you have made owing to their extreme thinness.

Treating the Sections with Iodine.—Remove the slide to the table. Run some iodine under the cover-glass with the help of blotting-paper, and then observe the preparation under the microscope.

Notice with what avidity the **starch grains** in the cells absorb the iodine and how the iodine turns them first **blue** and then **blue-black**.

In some of the cells just beneath the thin external cork layer, you may be able to find cubical protein **crystalloids**. There is, as a rule, not more than one in each cell, and, when one is present,

it often lies near the centre of the cell. A protein crystalloid can be identified by its straight edges, its sharp angles, its cubical form, and by gradually staining yellow with iodine.

Storage Starch Grains and Chloroplast Starch Grains contrasted. The starch grains of the potato tuber must be regarded **not as temporary starch** like the chloroplast starch grains, but as **storage starch**. They are stored away for the winter as reserve food material in a special magazine—a swollen underground stem—and, in the spring, they are converted into soluble sugar which is used to supply food for the growth of new shoots.

Storage starch grains differ from **chloroplast starch grains** in the following particulars : (1) they are stored often in special swollen organs or tissues, e.g. the hemispherical cotyledons of the Pea embryo, the endosperm of the grain of Wheat, and a Potato tuber, whereas chloroplast starch is only found in chloroplasts ; (2) they are often of relatively very large size ; (3) they are formed by leucoplasts instead of chloroplasts ; and (4) they are formed of sugar sent down from the leaves where the sugar has been manufactured from carbon dioxide and water by the chloroplasts ; whereas the starch grains in chloroplasts have been formed from sugar which the chloroplasts themselves have manufactured.

Make a drawing showing how the starch grains lie in a few of the potato-tuber cells. Head your drawing : **Storage Starch in the Tuber of a Potato**. Make each cell 1-2 inches in diameter.

CHAPTER XII

NUCLEAR DIVISION IN AN ONION ROOT. PROTOPLASMIC STREAMING IN A LEAF OF THE CANADIAN PONDWEED

Materials and reagents supplied :

- (1) A permanent preparation showing nuclei in the growing-point of an Onion root ;
- (2) The Canadian Pondweed plant, to be passed around for inspection ;
- (3) The Canadian Pondweed, a piece of stem bearing leaves ;
- (4) Iodine (dissolved in an alcoholic solution of potassium iodide) to be used for staining blue-black the starch grains contained in chloroplasts ;

- (5) Potassium nitrate, 10 per cent. solution, to be used for plasmolysing living cells.

A. THE NUCLEUS AND CELL-DIVISION IN THE GROWING-POINT OF AN ONION ROOT

Material.—You have been supplied with a very thin longitudinal section of the growing-point of an Onion root (*Allium Cepa*). The section is permanently mounted in Canada balsam.

Please take the greatest care of the slide supplied, as it is valuable and, if broken, cannot easily be replaced.

Remarks on the Making of the Preparation.—The sections that are cut with a hand-razor, such as you employ, are known as **hand-sections**. The root section supplied to you was cut by means of an instrument of great precision known as a **microtome**, and it can therefore be called a **microtome section**.

The **microtome**, as already remarked in Chapter VI, is an elaborate and costly instrument which works on the principle of the guillotine. By means of it one can cut a piece of root or a stem, etc., into a very large number of thin serial sections of any desired thickness. The section provided is mostly only one cell thick, and in places it may be even thinner.

When the Onion root was growing rapidly and new cells were being formed at the root tip, the end of the root was cut off and put into a **fixing solution** (possibly chrom-acetic acid). This solution killed the protoplasm in each cell and coagulated it without much altering its shape. Thus the protoplasm was **fixed** and **hardened** and so prepared for section-cutting.

The root was then **washed** in water to remove the fixative, then **dehydrated** (all the water removed) gradually with alcohol, and then passed through **xylol** into melted **paraffin wax**. The wax was allowed to cool and thus solidify, and then the **block of wax with the root tip embedded** in it was **cut** by a razor held in the microtome **into thin sections** (? about 10 microns = 0.01 mm. thick).

A paraffin section of the root-tip so cut was then laid on a slide previously smeared with albumen (to cause the section to adhere to the slide), and the slide was **warmed** to melt the wax. The wax was removed by xylol, and the xylol by alcohol. Then the section was stained with a blue dye known as **haematoxylin** or **logwood** (obtained from the heart-wood of *Haematoxylon*

campechianum, the Logwood, a tree species belonging to the family Leguminosae). The section thus stained blue was then washed with alcohol (to dehydrate it), and then with xylol (to remove the alcohol), and then it was mounted in a drop of **Canada balsam** (obtained from the resin-ducts in the wood and bark of **Abies balsamea**—the Balsam Fir of our forests). The balsam slowly dried up and hardened. Thus was obtained the so-called **permanent preparation** with which you have been provided.

Examination of the Section.—Examine the section with the low-power objective first. See that you have **correct illumination** (strongest possible light up tube from mirror, then light cut down with iris diaphragm to suit your vision; this gives the sharpest and clearest definition to any cells or structures to be examined).

Note that the cells at the root-tip are very **small** and rather **square** in outline, and that they are arranged in **longitudinal rows**.

Note further that the cells at the root-tip are **full of protoplasm** and therefore do not have a large central vacuole. Each **nucleus** occupies the middle of its cell, while the **cytoplasm** in which it is embedded fills up the rest of the cell-lumen.

Observe with the low-power objective (survey the whole length of the section) that **in the square-looking cells** at the root-tip the nuclei are all **rounded** in outline, whereas in the **elongated cells** which are developing into vascular-bundle tissue at some distance from the root-tip **the nuclei are distinctly elongated**. Evidently there is a correlation between the form of the cells and the form of the nuclei contained within them.

The **cell-walls** are excessively **thin** and as yet show no secondary thickening whatever.

Many of the **nuclei** are in the **resting condition**, i.e. not at the moment in a state of division. Such nuclei are rounded in outline and are surrounded by a thin **nuclear membrane**. Within this membrane is a clear substance called **nuclear sap** (karyolymph), which is not readily stained, and embedded in the nuclear sap is a **network** or **reticulum** composed of threads made up of linin in which are distributed particles or masses of another substance called **chromatin**. The **chromatin** of the linin thread-work is easily stained and it forms the conspicuous deeply-stained dotted part of each resting nucleus. In each nucleus there is one **nucleolus**, a rounded body much smaller than the nucleus

as a whole. The nucleolus disappears when the nucleus divides and may be utilised by the nucleus in its metabolic processes.

Make out the parts of the resting nucleus as described above.

Karyokinesis or Mitosis.—This is the phenomenon in which the nucleus divides with the formation of **chromosomes** which are split longitudinally. In the ordinary method of division the nucleus passes through many stages, and it is the whole complicated process which has received the names given above. Karyokinesis is of great importance because it brings about the division of all parts of the chromatin (the physical basis of the characters which are inherited) **equally** between the two daughter nuclei. There are various phases in karyokinesis: **prophase, metaphase, anaphase, and telophase.**

Prophase.—The first step in the division of the nucleus is the resolving of the linin network into a more or less continuous thread often called a **spireme**. **Look for nuclei in the prophase ;** you will know them by the threadwork having become much more conspicuous and clear than in the resting nuclei.

The linin threadwork next segments transversely to form portions known as **chromosomes**. The nucleolus and nuclear membrane then disappear, and a nuclear spindle is formed. **You will find here and there cells exhibiting chromosomes** and without a nucleolus or a nuclear membrane ; **but you will not perceive any spindles** as, unfortunately, the dye necessary to bring them out was not applied to the section.

Metaphase.—The prophase just described passes into the metaphase. When this stage is reached, the chromosomes, of which in the onion there are 16, become **arranged in a single plane at the equator of the spindle** (equatorial plane). By the end of the metaphase, each chromosome has been divided lengthwise into two daughter chromosomes. You may well have one or more nuclei in the metaphase in your preparation.

Anaphase.—The anaphase is the next stage after the metaphase. In the anaphase, one of each pair of daughter chromosomes travels to one pole and the other chromosome of each pair travels to the opposite pole. Thus the anaphase is the stage in which the **daughter chromosomes move along the nuclear spindle from the equatorial region to the poles.** You may possibly have in your preparation a nucleus in the anaphase of nuclear division. If so, you will see two groups of daughter chromosomes in one and the same cell.

Telophase.—When the chromosomes reach the poles, they collect into a more or less solid-looking mass. This is the beginning of the telophase. In this phase the daughter chromosomes become scattered to form a linin network like that in the resting nucleus, nuclear sap is produced, a nuclear membrane is developed, and a nucleolus appears. You may possibly have one or more nuclei in your section in telophase. If so, you will see **two nuclei bounded by nuclear membranes in one and the same cell** with no cell-wall as yet developed between them.

Formation of cell-walls.—As the chromosomes in a dividing nucleus become collected at the poles, a cell-wall begins to be formed across the middle of the cell. The wall begins as small thickenings on the spindle fibrillae in the equatorial region of the nuclear spindle. The spindle fibrillae spread out until the sides of the spindle reach the sides of the old cell-walls, and the thickenings of the fibrillae grow laterally until they fuse together to form **a continuous wall which stretches clear across the old cell**, thus dividing the cell into two daughter cells.

You may be able to find two cells which have just been produced from a single cell. The two cells will be alike and lie side by side, and their total length will be about equal to the length of a cell near-by which has not been divided recently.

Drawing.—Make a drawing showing: (1) a cell containing cytoplasm and a resting nucleus with its nuclear membrane, nucleolus and linin network; (2) a cell in which chromosomes are clearly seen, but no nuclear membrane or nucleolus; and (3) a cell in the anaphase or telophase, if you have observed it.

Each cell should be about an inch long, and you should represent some cells in at least two adjacent rows. The cell-walls are so thin that they should be represented by single thin lines.

B. PROTOPLASMIC STREAMING IN THE CELLS OF THE CANADIAN PONDWEED

The Canadian Pondweed, *Elodea canadensis*, is a submerged aquatic (grows entirely submerged in water) and is common in ponds and pools. It is easily grown in an aquarium in the laboratory or in a greenhouse, and it is often put into gold-fish bowls, where it uses for photosynthesis the carbon dioxide given off by the respiration of the fish. It has been introduced into

Europe, where it flourishes in ponds, canals, etc., as a very common water-weed.

A **living specimen** of the Canadian Pondweed showing the stem bearing leaves (three at each node) will be passed round to you for inspection.

Mounting a Leaf in Water.—A piece of a Pondweed stem bearing leaves has been supplied to you. Carefully **remove from it the whole of a single leaf** and mount the leaf in a drop of water. The removal of the leaf can be best effected by cutting it across its base with a pair of scissors or by putting the material on a slide and then severing the whole leaf by pressing across its base with a sharp scalpel or pen-knife.

Microscopic Observations.—With the **low-power** of the microscope note that a **mid-rib** or median vein runs up the central axis of the leaf from base to apex. The mid-rib is a **vascular bundle** consisting of several layers of narrow elongated conducting cells. Right and left of the mid-rib the **lamina consists of just two layers of cells**, both of which contain chloroplasts. Observe, by focussing up and down, that both these layers have their **cells arranged in longitudinal rows**. The cells of the upper layer are twice as broad as those of the lower layer, so that for every row of cells in the upper layer there are two rows in the lower layer. Between the two layers there are well-developed **intercellular spaces**. They are indicated by the presence of air in them, which makes them look like **longitudinal black streaks**. Notice these streaks. Here it may be remarked that **aquatic plants in general have large air spaces**. Elodea, therefore, is no exception to this rule.

The leaf is pointed at its apex, and its margin is **dentate**, just as in the Moss leaf already studied. Note that each tooth is due to the projecting of a single cell. Such a cell may be regarded as a simple **hair**. Note that the end of each hair is **red**, and that the redness is due to a red pigment situated **in the walls** and not in the cell-lumen.

The **green cells of the lamina** have the same general structure as those of the Moss leaf already studied. As usual, there is a thin dead **cell-wall** made up of the chemical substance called **cellulose**. Then in each cell, pressed against the cell-wall by the osmotic pressure of the cell-sap, there is a **primordial utricle** made up of colourless **cytoplasm** in which are embedded the **chloroplasts** and the **nucleus**.

The **chloroplasts** are **lenticular in form**, i.e. rounded or oval when seen in face view and flattened like a disk when seen in side view. Verify this statement by observations of your own. Here, as in the Moss, etc., the **chloroplasts multiplied** during the development of the leaf by **simple division**. Some of them in the **dumb-bell stage of division** may often be observed. Look for them. Each chloroplast is a fairly dense mass of living protoplasm coloured green by the pigment **chlorophyll**. In the middle of many chloroplasts you may be able to discern faintly one or more **starch grains**. These starch grains have been formed from surplus sugar manufactured by the chloroplasts from carbon dioxide and water. The chloroplasts must have produced the sugar, and also the starch grains formed from it, during the day, as the light of the sun is necessary for photosynthesis.

The Nucleus.—There is one in each cell, but in most of the cells it is completely hidden from view by the green chloroplasts. If you wish to see a nucleus, **look in the hair cells** at the leaf margin. Here one can very often see the nucleus as a colourless rounded mass of protoplasm lying at or near the base of the cell. In the large green cells in the middle of the lamina the nucleus can sometimes be seen on a lateral wall, and then it appears to be plano-convex—flattened on the side towards the wall and the convex on the other side. The nucleus is always embedded in the cytoplasm.

Protoplasmic Streaming.—Try to observe this remarkable exhibition of protoplasmic activity. The phenomenon is most frequently to be detected first in the elongated cells in the neighbourhood of the mid-rib, and usually nearer the injured leaf-base than the leaf-apex. Bring the **mid-rib** into the centre of the field with the low-power objective, and then examine its cells and the cells near it with the high-power. Focus up and down with the fine adjustment when examining the cells.

When a cell exhibits streaming, the cytoplasm making up the **primordial utricle** undergoes **rotation**. As the cytoplasm moves, it carries the green chloroplasts with it, and it is by the **movement of the chloroplasts** that streaming can be detected in the first place.

Usually streaming may be seen in some cells but not in others. You must **search for cells exhibiting the movement**. If you look only in one place, you may see no movement at all. **Examine cells all along the mid-rib**, particularly in its lower half. The

larger green cells right and left of the mid-rib often show some movement after some time, but it is the mid-rib cells which are usually the most active.

Remember that the **motion of the chloroplasts is entirely due to the motion of the cytoplasm** which carries in its streaming movements all the substances which are embedded in it. Sometimes the **nucleus** is carried along, but it usually goes much more slowly than the chloroplasts. Careful observation has shown that a very thin layer of the cytoplasm nearest to the cell-wall does not move at all, and that the layers nearest to the central vacuole move the most rapidly.

At first, when a leaf is removed from a plant and mounted, the cytoplasm in the cells is generally **quiescent**; but usually, **after a few minutes, motion appears**. As a rule motion may be first detected in some of the long cells of the mid-rib. At first it is very slow and irregular; a single chloroplast or a group of chloroplasts is slightly moved, then the motion may extend to a larger mass of the cytoplasm, and soon a stream is started which carries the chloroplasts, and perhaps the nucleus, slowly **up one side of the cell and then down the opposite side**. The streaming quickens its pace and soon attains a maximum. While all this has been taking place in one cell, streaming may have begun in other cells, and soon many cells may exhibit the phenomenon. Then the scene within the field of view of the microscope is a very busy and striking one.

When streaming is sluggish, it may often be accelerated by warming the preparation, e.g. by putting the slide on a hand. If, after some time, you cannot observe streaming, **request a demonstrator** to look at your preparation or to give you another leaf.

The streaming only takes place in living cells. It is a phenomenon of life and is a good example of spontaneous protoplasmic movement.

Make a sketch of a single cell (or more than one cell if you wish), not less than about two inches long. Indicate the **cell-wall**, the **primordial utricle**, containing **chloroplasts**, the **nucleus** if you have seen one, and the large central **vacuole**. Indicate by means of **arrows** the direction of streaming around the cell (put the **arrows** in the vacuole).

Is there any Starch in the Chloroplasts?—Remove the cover-glass, suck away the water with blotting-paper, add a drop of

iodine, and then add a cover-glass. Examine with the microscope.

If starch grains are present in the chloroplasts, they will become blue-black, as in a Moss leaf. If starch grains are present, what is their shape? Are they relatively smaller or larger than those you saw in the Moss chloroplasts?

Remember that here, as in chloroplasts in general, the starch grains are the first visible products of the very important physiological process called **photosynthesis**.

If you have observed starch grains in the chloroplasts, put them in the **chloroplasts in your drawing** just made.

To Isolate some Chloroplasts.—Remove the cover-glass from the preparation, **tear the leaf to bits** by means of a pair of needles or a needle and knife, and then replace the cover-glass.

Some of the chloroplasts will have now been torn out of the cytoplasm in which they were embedded and will be **lying free in the mounting fluid**. Look for some of these isolated chloroplasts. You will know them by their size, shape, colour, and starch-grain content.

Is not the separation of the chloroplasts, as just effected, evidence that the chloroplasts are made of much denser and firmer protoplasm than the cytoplasm?

Sketch some of the isolated chloroplasts on a large scale. Show their exact shapes, and the shapes of the starch grains which they contain.

Plasmolysis and De-plasmolysis.—Take a fresh leaf or a piece of it, and lay it on a slide. Then add a drop of 10 per cent. potassium nitrate. Then observe first with the low-power objective in order to find cells in which **plasmolysis** is beginning, and then with the high-power in order to observe details of the contraction of the primordial utricles away from the cell-walls.

After the cells have become well plasmolysed, **de-plasmolyse them by running water under the cover-glass** with the help of blotting-paper. The water will make its way through the cell-walls and will replace the potassium nitrate solution. As soon as water, instead of the salt solution, comes to bathe the outer surfaces of the contracted primordial utricle in any cell, **de-plasmolysis** begins. Observe its progress. Water passes into the vacuoles by osmosis, and the vacuole therefore increases in size. As the vacuole increases in size, its cell-sap presses out the primordial utricle, causing it to expand, and soon the primordial

utricle is again pressed against the cell-wall. Thus, in deplasmolysis, the original position which the primordial utricle had before plasmolysis took place is once more restored.

CHAPTER XIII

PROTOCOCCUS. THE EPIDERMIS OF A MONOCOTYLEDON

Materials and reagents supplied :

- (1) *Protococcus viridis*, living plants on bark, obtained from England ;
- (2) A leaf of a Hyacinth ;
- (3) Iodine.

A. PROTOCOCCUS VIRIDIS

Multicellular and Unicellular Plants. — Flowering Plants (Phanerogams), e.g. Sunflower, Wheat, Poplar, are made up of millions of cells and are therefore **multicellular plants**, but there are some Flowerless Plants (Cryptogams), e.g. *Protococcus viridis* and the Yeast plant, which consist of a single cell only and are therefore **unicellular plants**. The first unicellular plant to be studied in this course will be *Protococcus viridis*. For making acquaintance with the lower forms of plant life, *Protococcus* is a classical object, universally described in botanical text-books and well known to all botanists.

Protococcus viridis is a unicellular plant which is found growing in large colonies on the bark of standing trees and of logs, and on walls, fence-posts, rocks, etc., in England, British Columbia, and many other parts of the world where the climate is moist. It does not occur in Manitoba, as our climate is too dry. The material—bark and sticks coated with green colonies of *Protococcus viridis*—provided for this laboratory period was collected in England and arrived a few weeks ago. Since it came to hand it has been kept moist and has been exposed to the light.

Macroscopic Observation of a *Protococcus* Colony.—With the naked eye, examine the piece of bark or wood provided. Note that its surface bears a thin layer of green matter. Does the colour suggest the presence of chlorophyll ? The green matter consists of tens of thousands of minute green plants, each one of

which is so small that it cannot be seen individually with the naked eye. These microscopic plants all belong to *Protococcus viridis* (*viridis* = green), and this species is included in the great group of flowerless plants known as the **Algae** (sing. *Alga*; plural, *Algae*). Most Algae live entirely submerged in the water of lakes, ponds, rivers, seas, etc.; but *Protococcus viridis* lives on the surface of bark, etc., so that it is only covered with water when it rains. The little plants grow and multiply during moist weather. When dry weather comes, they soon dry up and become quiescent, but they retain their vitality in the desiccated condition for weeks or months until it rains again. As soon as they once more become covered with a film of rain-water, they quickly resume their vegetative growth and begin to multiply by cell-division.

To Make a Microscopic Preparation of Protococcus Plants.—Place a drop of water in the middle of a slide. With a needle remove a very little of the green substance from the piece of bark or wood supplied and put it in the drop. Tease out the substance with the help of two needles or a needle and knife, so as to separate from one another as much as possible the tiny Protococcus plants of which it is composed. Remove any bits of bark or hard particles which might raise up the cover-glass too much. Add a cover-glass. If necessary, run more water under the cover-glass, so as just to fill the space there. Examine the preparation first with the low-power objective and then with the high.

Observations with the Microscope.—Under the microscope the Protococcus plants are, seen as very small green cells, sometimes single, but more often aggregated into groups or colonies of two, four, or more cells each. **Each cell is a Protococcus plant** which can live an independent life.

With the **low-power objective** notice how very small the Protococcus plants are, compared, for example, with even single cells of a Moss leaf or of the epidermis of an Onion scale. The diameter of the field of view with the low-power objective is about 1.75 mm., and the diameter of a single Protococcus plant is only about 0.01 mm. ($= \frac{1}{100}$ mm. $= \frac{1}{2500}$ inch).

With the **high-power objective**, move your slide about (arms on table, finger and thumb of both hands on the ends of the slide) and find favourable places for study. In other words, explore your preparation sufficiently. Find **isolated Protococcus plants** and **colonies of two, four, or more individuals**. Usually colonies

of four plants or less united to one another are of very common occurrence. Is this so in your preparation ?

Observe that individual plants, if isolated, are almost spherical, but that in the cell-groups of two or four individuals each cell is more or less flattened on the sides in contact with other cells.

Observe that each plant or cell has a fairly thick colourless **cell-wall**. This wall is made of cellulose, a fact that has been ascertained by observing that chlor-zinc iodine causes the walls to become violet. The cell-contents consist of living protoplasm of three kinds : (1) **cytoplasm**, (2) a **nucleus**, and (3) a much-lobed **chloroplast** which is embedded in the cytoplasm and is close under the cell-wall. The contents of each cell have an almost uniform green appearance owing to the large size of the single chloroplast, but here and there in a cell portions of the colourless cytoplasm may sometimes be perceived. The nucleus cannot be seen without the help of reagents. Iodine may be used to bring it into view.

Reproduction in Protococcus.—The usual method of multiplication, i.e. of increasing the number of Protococcus individuals, is a **vegetative** one. All that happens is that a single cell grows in size, undergoes cell-division, and then the daughter-cells gradually separate from one another. One plant may thus produce a colony of two plants. Two such plants, before separating from one another, often undergo cell-division, in their turn producing daughter-cells. Thus colonies of four cells frequently come into existence. The four cells of a colony gradually separate from one another or, if undisturbed, they may continue the multiplication process and form colonies of 8, 16, or more cells loosely attached together. In this way the extensive green colonies on bark, etc., gradually come into existence. The **dispersion** of Protococcus plants in nature and their spread from tree to tree, etc., is probably due in large measure to the wind, which may detach them from the bark when they are dry, but it is certainly often effected by birds, to the feet of which Protococcus plants readily adhere.

Do not fail to comprehend that **each Protococcus cell is a complete plant** that by itself can carry on all the functions of life. **Protococcus viridis** may be described as one of the simplest and most lowly of all green plants. What green plant could well be simpler in its organisation than one which shows no division into stem, leaf, or root and which consists of a single spherical microscopic cell ?

Make drawings showing: (1) a single isolated *Protococcus* plant, (2) a colony of two plants, (3) a colony of four plants, (4) any other colony that may interest you. Probably some of the colonies seen will be just coming into existence and showing early stages in cell-division.

A single plant may be drawn about one-quarter of an inch in diameter. Then a colony of four individuals would have a diameter of about half an inch (= 12.5 mm.). This would be equivalent to representing the *Protococcus* plants with a magnification of about 1,250 diameters. At the top or side of a drawing made on this scale you should indicate the magnification with the multiplication sign followed by the number 1,250. Here, as always, you must write above your drawings what it is you are attempting to represent. In this case it will be ***Protococcus viridis***—a green Alga. Indicate in writing: a single plant with its cell-wall and chloroplast, a two-celled colony, a four-celled colony, and a cell or colony in process of cell-division.

B. THE EPIDERMIS OF A MONOCOTYLEDON

General Remarks.—The epidermis is one of the **primary plant tissues**, and it consists of a single layer of cells which bounds a stem or leaf on its exterior. One of its chief functions is to protect the underlying tissues from mechanical injuries and from too rapid a loss of water.

In **Monocotyledons**, e.g. Grasses, Sedges, Lilies, Daffodils, etc., the **leaves** are usually much **elongated** and **parallel-veined**; while in **Dicotyledons**, e.g. Sunflower, Roses, Poplars, etc., the **leaves** are usually fairly **broad** relatively to their length and are **net-veined**. The shape and arrangement of the cells of the epidermis of Monocotyledons and of Dicotyledons are correlated with the shape and mode of venation of the leaves. We shall attempt to verify this conclusion by comparing the epidermis of a Hyacinth (a Monocotyledon) with that of a Geranium (a Dicotyledon).

The Hyacinth.—The Hyacinth is a bulbous plant which flowers in the spring at the expense of the reserve food materials (starch and inulin) which were stored up in the thick, fleshy, food-containing scale-leaves of the bulb in the previous summer. Owing to the beauty of its flowers (white, red, or blue), the time at which it blossoms, and the ease with which it can be procured

and grown, the Hyacinth in the early months of the year is a favourite house-plant. The bulbs are kept moist in well-aerated soil in a cool place in the dark until they are well-rooted. Then they are brought into a warm greenhouse or room and exposed to the light, whereupon they rapidly develop their flowers in a normal manner.

Macroscopic Observations on a Hyacinth Leaf.—Examine the leaf supplied to you already or which will be passed around to you. Notice that it is much elongated and that its two edges are nearly parallel to one another, also that its lower half is semi-cylindrical, its upper half concavo-convex, and its apex blunt and somewhat resembling the end of a boat.

The **concavo-convex form** of the Hyacinth leaf is **an important factor in giving the leaf mechanical rigidity**. To prove this by **experiment**, take a sheet of paper and with a pair of scissors or a pen-knife cut out a piece of paper of about the same shape as the Hyacinth leaf when flattened. Now take hold of the piece of paper at one end and attempt to hold it in the air in a plane parallel to that of the top of the table. Notice that so long as the piece of paper is **flat** it cannot be held out horizontally as it is too flexible: it sags downwards by its own weight. Now **bend the piece of paper until it has the same form as that of a Hyacinth leaf**, i.e. until it is concavo-convex. Then hold it by its base in the air. You will now find that the rigidity of the piece of paper has been increased to such an extent by folding that the piece of paper can be readily held in a horizontal position. May we not conclude from this experiment that the shape of a Hyacinth leaf, from the point of view of vegetable mechanics, is a matter of considerable importance?

Observe that the leaf has **parallel veins** and **no mid-rib**. This is characteristic of most monocotyledonous leaves.

Notice that the leaf is chlorophyllaceous green for most of its length, but that **at its base it is a very pale-green or even colourless**. Why is the base of the leaf so pale? The leaf elongates not by growth at its apex but by **intercalary growth at its base**. The zone of growth being at the leaf base, the lowest part of the leaf is the youngest and last-formed part. It is pale-green because the green colouring matter, chlorophyll, develops only under the action of light and its development takes some time. The very base of each leaf (not included in your specimen) is colourless because it is inside the bulb and therefore in the dark.

Notice, further, that the leaf is **translucent**, i.e. allows light to filter through it. To observe this, hold the leaf against your eye and look toward a window. Evidently, the leaf is so constructed that every chloroplast in it will obtain sufficient light to carry on the work of photosynthesis.

Make a sketch of the leaf showing its form. Indicate the **parallel veins**, the **apex**, and the **base**. You should now be able to make this drawing fairly rapidly.

To Make a Preparation of the Lower Epidermis.—Put a drop of water in the middle of a slide. Take a leaf and notice which side is the under side. Now wrap the leaf with the under side toward you over the first finger of the left hand and hold it in place with the thumb and second finger. Next take a sharp knife, scalpel, or razor and make a transverse shallow incision in the leaf so as to cut through the epidermis for a distance of one-eighth to one-quarter of an inch. Then with the help of your fingers and a scalpel or pen-knife (or by employing forceps) **peel off a strip of epidermis**. As you pull away the strip from the leaf you may find that the strip is green, owing to green sub-epidermal cells remaining attached to the epidermis; but, usually, as the strip gets longer, it becomes more or less colourless. When the strip is quite colourless it consists entirely of epidermis. If you do not succeed in obtaining a suitable strip at the first attempt, try again. Strips are most easily obtained from the lower half of the leaf.

When the required strip of epidermis has been pulled up, cut it away from the rest of the leaf with the help of a pair of scissors or a sharp scalpel or a razor. Then lay the piece of epidermis in the drop of water on the slide with what was the **outer side facing upwards**. If the piece of epidermis is folded over itself, it must be flattened out with the help of needles. When the piece of epidermis is well spread out, put another drop of water over it and add a cover-glass. Then observe the preparation first with the low-power objective and then with the high.

Observations with the Low-power Objective.—Note that the Hyacinth epidermis, like that of the scale of an Onion, consists of a **single layer of cells**, that all the cells are **arranged in rows**, and that **most of the cells are much elongated**. Is the long axis of each epidermal cell in a normal leaf parallel to the long axis of the whole leaf? If so, we must conclude that there is a correlation between the shape and arrangement of the epidermal cells and the shape of the leaf.

The most conspicuous parts of the epidermis are the **stomata**. A **stoma** (derived from the Greek word meaning a *mouth*, plural *stomata*; commit this to memory) is an **intercellular passage** perforating the epidermis and bounded by two elliptical but somewhat curved cells called **guard-cells**. The intercellular passage or air-space is known as the **stomatic cleft**, and **it is via the stomatic clefts that gases pass into or out of a leaf**. During the hours of light, carbon dioxide passes into green leaves *via* the stomata and oxygen passes out *via* the stomata, the two gases diffusing in opposite directions. Under certain conditions, e.g. in bright sunlight, the two guard-cells of each stoma swell up and enlarge the stomatic cleft, while under other conditions, e.g. of drought, the guard-cells shrink and close up the stomatic cleft. The loss of water by a leaf, a phenomenon known as **transpiration**, is chiefly due to the escape of water vapour through the stomata. Notice that the **guard-cells** of the stomata alone **contain chloroplasts**, all the other cells being colourless. If there are any sub-epidermal cells attached to the under side of the epidermis, they will appear rounded and also green owing to their containing numerous chloroplasts.

Notice with the low-power objective the **distance the stomata are apart and how they are arranged** in the epidermis as a whole. Also observe that the epidermal cells, other than the guard-cells, are very much elongated and colourless. **Look for cross-walls** separating adjacent cells in a single row. Are these cross-walls strictly transverse, or are they oblique?

With the high-power objective study the epidermis in detail. Bring a stoma into the middle of the field of view and make out its parts. Its central elongated **stomatic cleft** may look black owing to the presence of **air** held in it between the **two guard-cells**. Make out the exact shape of each of the two guard-cells and how they are attached to one another at their ends. Notice that at each end of the stomatic cleft there is a definite **cell-wall** separating the two guard-cells from one another, so that each guard-cell is somewhat sausage-shaped. Notice the numerous rather small rounded green **chloroplasts** embedded in the cytoplasm of each guard-cell. In these chloroplasts there may be some minute grains of starch.

Observe carefully the position of the stoma in relation to the adjacent cells. At each end of the stoma two walls of a single elongated epidermal cell are attached, one to the wall of one

guard-cell and the other to the wall of the other guard-cell. **Look for these walls.** Does or does not the long axis of the stomatic cleft coincide with the long axis of the cells of a row of ordinary epidermal cells? Determine this point before attempting to make a drawing.

When the epidermis was torn away from its subjacent tissues its cells were subjected to such a violent strain that they were killed. Hence you will find that the **cytoplasm** in the ordinary elongated cells is **shrunken away from the cell-walls**. Often also the **nuclei** may be seen as distorted worm-like objects, very different in appearance from what they were like when the cells were living. The cell-walls of the elongated cells are quite straight under normal conditions but, now that these cells are dead, you may find the walls have become somewhat wavy or corrugated.

Make a drawing of the epidermis about three inches broad and four to six inches long. **Each stoma should be made about half an inch in diameter** and all the other structures should be drawn to the same scale. Show two or three stomata scattered singly here and there in the rows of ordinary much elongated cells. Indicate with names the **guard-cells** and their **chloroplasts**, the **stomatic cleft**, the **cell-walls**, the **ordinary epidermal cells** and their **disorganised cytoplasm** and **nucleus** (if you have observed it). As usual, write above your drawing what it is meant to represent.

To Estimate the Number of Stomata on a Leaf.—Turn on the low-power objective. Place in the field of view a piece of epidermis that fills it completely. Count the number of stomata in the field of view.

Now **estimate the area of the field of view**. The area enclosed by a circle = πr^2 , i.e. equals the square of the radius multiplied by 3.1416 (approximately $\frac{22}{7}$). To find the radius of the field of view, measure the diameter and then divide by two. To measure the diameter employ the **white paper measuring-rod** with which you are provided. Place it in the field of view and estimate the diameter in mm. as nearly as possible. This diameter may vary in different microscopes from about 1.5 to 2 mm. Having found first the diameter and then the radius of the field of view, calculate the area of the field of view by means of the formula already given. The area will be in square mm.

Now **calculate how many stomata there would be on one square centimetre**. Having found the number of stomata on one square cm. find roughly **the area of a typical leaf**, taking both sides into

consideration. Then calculate how many stomata the leaf has approximately.

Record your calculation in your book. Set down: the **diameter of the field of view** with the low-power objective, the **number of stomata** you saw in the field of view, the total **area** of the two sides of a **typical leaf**, and the approximate **number of stomata** which you have estimated to be **present on a typical leaf**.

The stomata of a Hyacinth leaf are relatively large and widely scattered. Many leaves, e.g. those of a **Geranium**, have much smaller and more numerous stomata. Thus it has been calculated that a medium-sized **Apple leaf** may have as many as 400,000 stomata, but in this instance all the stomata are on the under side of the leaf and there are none on the upper side. A **Banana leaf**, which has a lamina sometimes 15 feet long and 3 feet wide (45 sq. ft. on each side), must have many millions of stomata in its epidermis.

CHAPTER XIV

THE YEAST PLANT. THE EPIDERMIS OF A DICOTYLEDON

Materials and reagents supplied :

- (1) Brewer's yeast ;
- (2) A Fleischmann's yeast-cake ;
- (3) Iodine ;
- (4) A leaf of a Geranium.

A. THE YEAST PLANT

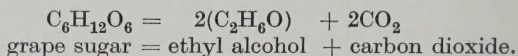
The Yeast Plant is a fungus of peculiar interest to man, because it is the exciting cause of alcoholic fermentation in saccharine solutions. There are several species of Yeast, of which two may here be mentioned :—

- (1) *Saccharomyces cerevisiae*—the Bread and Beer Yeast Plant, kept in cultivation in breweries, yeast-factories, etc., and
- (2) *Saccharomyces ellipsoideus*—the Wine Yeast Plant, which grows wild in nature and often settles on the skins of grapes in vineyards, etc.

To-day it will be our business to study the Beer Yeast Plant.

Saccharomyces cerevisiae.—This micro-organism is used by bakers for raising dough and also by brewers for making beer, ale, porter, etc. The wild species occurs all the year round in the soil and during summer and autumn on sweet juicy fruits, etc. It is dispersed by the agency of wind, rain, wasps, etc. Its cultivated varieties, like the cultivated varieties of Wheat and other cereals, are not known wild, but are kept in existence by man. The Yeast Plant has been continuously employed in the bread-making and beer-making industries for many centuries.

The Material Supplied.—This consists of a pale sugary fluid contained in a glass vessel. It was obtained yesterday from a neighbouring brewery, and more sugar was added to it in the laboratory. Examine the fluid **macroscopically**. **Is the fluid clear or turbid?** If it is turbid, there must be numerous particles suspended in it. These particles are hundreds of thousands of minute unicellular yeast plants. What is the colour of the fluid? Judging by the colour test, does or does not the Yeast Plant contain chlorophyll? **Are any minute gas bubbles being formed in the fluid?** If numerous gas bubbles can be seen gradually forming and rising to the surface, it is evident that **alcoholic fermentation** of the sugar is in progress, i.e. **the sugar is being split up by the action of the yeast plants into alcohol and carbon dioxide**. So much carbon dioxide is being formed that the fluid has become super-saturated with it, the result being that what cannot be dissolved gathers into gas bubbles which escape from the fluid by rising to the top, forming froth there for a time, and then bursting. The froth on fermenting sugary solutions and on beer, etc., is entirely due to the escaping carbon dioxide gas. The fermentation process is caused by the yeast plants in an indirect manner as follows. Each yeast plant excretes into its vacuole an **enzyme** (an organic catalytic agent) called **zymase**. The sugar in solution in the sugary medium makes its way into the vacuole and there it is acted upon by the zymase and split up into alcohol and carbon dioxide. Other by-products are produced, e.g. glycerine and succinic acid; but, in the main, the process of alcoholic fermentation can be represented by the following chemical equation:



When yeast plants begin to ferment a sugary fluid, they quickly

use up all the oxygen present in it, while the fluid in which they are immersed becomes saturated with carbon dioxide. Yet the yeast plants continue to grow and multiply. Evidently, **yeast plants are able to live either aerobically**, i.e. in the presence of free oxygen which they can use up for respiration, **or anaerobically**, i.e. in the complete absence of free oxygen under which condition respiration is of course impossible. In the fluid supplied to you the yeast plants are living anaerobically. Yeast plants, in being able to live and flourish for a very long time without free oxygen, differ from all Flowering Plants and from all other Cryptogams except certain species of Bacteria which are also anaerobic.

The term **yeast** is often applied to a **liquid culture** of yeast plants undergoing fermentation (such a culture as you have before you) or to **moist compressed yeast plants** done up as soft cakes in tinfoil (Fleischmann's compressed Yeast) or to **dry cakes of yeast plants** (Gillett's dried Royal Yeast cakes). Remember, therefore, that whereas **yeast** is a commercial or domestic product, a **Yeast Plant**, in the scientific sense, is a microscopic living organism.

To Make a Microscopic Preparation of Yeast Plants.—Place a drop of water on a slide and add to it a very little of the fluid from the yeast culture. Stir with a needle. Add a cover-glass. Examine first with the low-power objective and then with the high.

Observations with the Low-power Objective.—Notice that the yeast plants scattered through the preparation are very numerous and extremely small. The diameter of the field of view with the low-power is, as you found last week, about 1.6 mm. Compare the length of a single yeast plant with the diameter of the field of view. Is it not clear that a single yeast cell is about one-hundredth of a mm. in length? Yeast plants and *Protococcus* plants are of about the same size. Note the general **absence of the green colouring matter chlorophyll** from all the plants.

Observations with the High-power Objective.—Observe the **shape** of the yeast plants. Are the plants spherical or oval (egg-shaped)?

Notice that each yeast plant consists of a single cell and is therefore **unicellular**.

Examine one of the larger plants as carefully as possible. It is bounded by a **cell-wall** and contains living protoplasm. Most

of the protoplasm is **cytoplasm**, but a **nucleus** is also present. The nucleus cannot be seen clearly without the application of staining reagents, but it consists of a chromatin network enclosing the **central vacuole**. Look for this large rounded central vacuole and remember that in the Yeast Plant it is considered as being part of the nucleus—a very exceptional arrangement. In addition to the central vacuole, **other vacuoles** may sometimes be present in the cytoplasm.

Around the wall of the central vacuole you may be able to see some very small rounded **brightly shining particles**. Some of these are **fat globules** and others **volutin** (? a reserve protein).

Note the **absence of chloroplasts**. Evidently the Yeast Plant is **colourless** or nearly so. **Algae**, e.g. *Protococcus* and *Spirogyra*, contain chloroplasts, but **Fungi**, e.g. Mushrooms and Toadstools, lack them entirely; and this is one of the most important differences between these two groups of plants. The Yeast Plant has no chloroplasts and therefore cannot be an alga. As a matter of fact **it is a fungus**. Just as *Protococcus viridis* is a unicellular Alga, so *Saccharomyces cerevisiae* is a unicellular Fungus.

Each yeast plant is surrounded by a **thin dead cell-wall**, in this respect resembling the cells of *Protococcus* and of the Flowering Plants; but the wall **does not contain any cellulose**. In lacking this characteristic vegetable compound Yeast resembles most Fungi. What the Yeast cell-wall is made of no one seems to know, but neither cellulose nor chitin have been detected in it. The cell-wall can best be seen in dead yeast cells. **Look about for such dead cells**. There may be a few of them present in your preparation. They are shrunken and their protoplasm is contracted away from the cell-wall, thus exposing the latter to clear view.

Reproduction in the Yeast Plant.—The common method of reproduction is by **vegetative budding**. When yeast plants are in an active state of growth, each of them gives rise to a tiny outgrowth (sometimes two) at one end of the cell. This outgrowth or **bud** gradually increases in size and is soon cut off from its parent plant as a separate yeast plant. In its turn, it may grow to full size and bud. This method of reproduction, i.e. of multiplying the number of Yeast individuals, is known as **gemination** (*gemma* = a bud) or **pullulation**. It differs from ordinary cell-division (observed in *Protococcus*) in that the parent cell does not divide into two cells by means of a wall

formed across its centre but, instead, the daughter-cell grows out as a bud from the parent-cell.

Look for yeast plants showing a tiny bud at one end. The buds separate from their parent plants usually before they have grown to be half the size of the parent. One can tell young yeast plants from old yeast plants in a preparation by their size. **Notice this difference in size by actual observation.**

Make a drawing showing : (1) several large isolated full-grown yeast plants, (2) some small, isolated half-grown yeast plants, (3) a yeast plant or two in the act of budding, and (4) a dead yeast plant. In a single large yeast plant, indicate the **cell-wall**, the **cytoplasm**, the central **nuclear vacuole**, and the **oil globules** or **volutin particles**.

The Action of Iodine on Yeast Plants.—Run some iodine under the cover-glass and observe its effects. Notice that the iodine instantly **kills the yeast plants**, thereby causing them to become somewhat shrunken. Also observe that the **cytoplasm turns yellow**, thus showing that it is made up of proteins. Notice further that many cells turn **brown** either as a whole or locally. This colouration is due to the presence of **glycogen** in the cells and, particularly, in certain vacuoles. Glycogen is a carbohydrate closely allied to starch, but it **stains brown with iodine** and not blue. Glycogen occurs in the cells of many fungi and it is also stored up in the human liver as a reserve food material that can readily be converted into sugar.

Notice that there is nothing in the yeast plants that stains blue with iodine. From this we may conclude that yeast plants **do not manufacture starch**. While starch is present in many Algae, it is never found in any of the Fungi.

Fleischmann's Yeast.—Fleischmann's Yeast in the form of small cakes is manufactured by a New York firm and supplied daily to firms and individuals requiring it. It is employed for bread-making in bakeries and private houses, and it is also eaten as a food. On account of the large amount of proteins, etc., in the yeast plants, it is highly nutritious and, in addition, it contains a water-soluble vitamine, known as **Vitamine B**, a valuable growth-promoting substance necessary for the metabolism of the higher animals, including man (it is anti-polyneuritic and anti-beri-beri).

If Fleischmann's Yeast, which is sold in **moist** cakes, is mixed into a sugary solution, e.g. 10 per cent. cane-sugar (30 grams of

yeast to 500 cc. sugar solution), the yeast plants immediately begin to ferment the sugar, and within an hour one can see great numbers of minute carbon-dioxide bubbles rising to the surface of the liquid and forming a froth there. When **dry** yeast cakes (Royal Yeast) are used instead of moist ones (Fleischmann's Yeast) one has to wait longer for active fermentation of the sugary solution, as the yeast plants in dry cakes are at first less active than those in moist cakes.

Microscopic Examination of Fleischmann's Yeast.—Put a drop of water on a slide. With a needle add to it a very little of the substance of the yeast cake. Stir up the drop. Add a cover-glass, and examine the preparation under the microscope.

Is a Fleischmann's yeast cake made up entirely of yeast cells or does it contain in addition a certain amount of starch? Do the yeast plants look as though they are living? Do they resemble the yeast plants obtained from a brewery? The iodine test may be used to decide whether your preparation contains any starch grains. **Record in your book** a statement of what you have found in Fleischmann's Yeast.

B. THE EPIDERMIS OF A DICOTYLEDON

In Chapter XIII it was pointed out that the shape of the leaf together with the arrangement of the epidermal cells differs in Monocotyledons and Dicotyledons. We have already studied a monocotyledonous leaf (Hyacinth). To-day we shall study a dicotyledonous leaf (Geranium). When this has been done, we shall have a basis for comparing the shape of the leaf and the arrangement of the epidermal cells of a typical Monocotyledon with those of a typical Dicotyledon.

The Geranium.—The so-called "Geranium" of our greenhouses is included in the natural family of Flowering Plants known as the **Geraniaceae**. However, it does not belong to the genus *Geranium* but to the genus **Pelargonium** and its specific name is *Pelargonium zonale*. In this Chapter we shall follow the nomenclature of the horticulturists and refer to our plant as a *Geranium* and not as a *Pelargonium*.

Macroscopic Observations on a Geranium Leaf.—A leaf of a *Geranium* has been supplied to you. Be careful, while handling it, not to crush the hairs upon it more than is necessary, as later on the hairs are to be studied under the microscope.

Notice that the leaf is divided into a cylindrical **petiole** and a flattened **lamina**. Notice that the lamina is not parallel-veined but **net-veined**. Net-veining is characteristic of the leaves of Dicotyledons. Notice also the **shape** of the lamina. The lamina is not elongated as it is in Monocotyledons, but it is more or less rounded (orbicular). **Which side of the lamina is the greener**, the upper or lower? Record your answer in your book. Is the lamina **translucent**?

The leaf has a great many **epidermal hairs**. With the naked eye examine the petiole and both sides of the lamina and **find out where the hairs are situated**.

Make a drawing of the Geranium leaf showing the **petiole**, the **lamina**, and the **chief veins**. As usual, indicate what the drawing is meant to represent and the names of the parts.

To Make a Preparation for Examining the Lower Epidermis.—Put a drop of water on the middle of a slide. Then, using the method already applied to the Hyacinth leaf (Chapter XIII), with a sharp pen-knife or scalpel and a finger strip away a small piece (3–6 square mm.) from the under side of the lamina of the Geranium leaf. It is impossible to strip the epidermis away as easily as in the Hyacinth leaf, but a very little bit of epidermis is all that is required. Part of your preparation, or even most of it, may be simply a green piece of leaf, but at its edge there should be **a piece of epidermis free from the sub-epidermis**. Mount the preparation in the drop of water on the slide and see that the **outer surface looks upwards**, as it is the outer surface that is to be observed. To get rid of air around the preparation, fill the space beneath the cover-glass with water, then raise and lower one edge of the cover-glass a time or two.

Observations with the Microscope.—First with the low-power objective and then with the high, find a piece of epidermis and examine it in detail.

Note that the **stomata** are small and numerous, and are **scattered irregularly** in the epidermis, and that the **ordinary epidermal cells** have curiously **wavy** or **corrugated lateral walls**, so that each cell is something like a piece of a picture-puzzle. The corrugations in the lateral walls doubtless serve to strengthen the epidermis mechanically. Compare the epidermis of the Geranium leaf with that of the Hyacinth leaf studied in a previous lesson.

Notice that the epidermis of the Geranium leaf is colourless except for the two guard-cells of each stoma which are green

owing to the presence of **chloroplasts**. Notice that, just as in the Hyacinth, **each stoma** consists of **two guard-cells** surrounding an elongated **stomatic cleft**. Notice further that the ordinary epidermal cells are not much larger than a stoma and that they fit tightly together without any intercellular spaces being present between them.

With a little care, at the edge of the green part of the preparation where the air in the intercellular spaces of the sub-epidermal tissues has escaped and been replaced by water, you should be able to observe the epidermis covering the green sub-epidermal tissues.

Make a drawing showing the epidermis. This should include **several stomata** with their long axes turned in different directions and a number of **ordinary epidermal cells**. The drawing may be made some three inches long and three inches wide. Each **stoma** with its pair of **guard-cells** should be made about 0·5 inch long and everything else drawn in proportion. Indicate what the drawing is and what are its parts.

To Make a Preparation showing the Epidermal Hairs.—The epidermal preparation already made may possibly be of use in enabling you to see something of the epidermal hairs, but the hairs can always be seen by making preparations as follows:—

(1) **Cut a small piece** (about 1–2 mm. wide and 5 mm. long) **off the edge of the lamina** with a pair of scissors or a sharp knife, and mount it in water under a cover-glass. The hairs at the extreme edge of the leaf will then be seen projecting out into the water.

(2) **Take a petiole**, cut it across transversely with a pen-knife so as to sever it from the lamina, avoid crushing the hairs with the fingers, and then with a razor cut some fairly thin, but not too thin, **transverse sections** (cross-sections) through it. Mount the sections in a drop of water, add a cover-glass, and examine with the microscope in the usual way. The hairs will be seen projecting radially away from the epidermis into the water.

Description of the Epidermal Hairs as seen in a Transverse Section of the Petiole.—The epidermis of the leaf of the Geranium bears two distinct types of hairs: (1) **conical** and (2) **capitate** (with a swollen head-cell).

(1) The **conical hairs**, which may be called **bristles**, are either (a) delicate and unicellular, or (b) stouter and multicellular with one or two cross-walls (**septa**), or (c) very long and tapering, and

multicellular with several cross-walls and a distinctly swollen base.

(2) **The capitate hairs**, which may be called **glandular hairs** (since their head-cells usually secrete a peculiar fluid), are: (a) short glandular hairs with a round head-cell on a short stalk 2-3 cells long, or (b) glandular hairs with an obovate head-cell set somewhat obliquely on a short stalk, or (c) doubtfully glandular hairs with a pear-shaped head-cell on a long 2-3-celled stalk.

The outermost layer of the outer wall of the epidermis as a whole is a special layer, very impervious to water, known as the **cuticle**. It covers not only the ordinary epidermal cells and the guard-cells of the stomata but also the hairs (which are epidermal products). **The cuticle may be seen on the exterior of the walls of the large cells of the very long bristle hairs.** Look for it. It may also be detected on the exterior of the ordinary cells of the epidermis in thin sections.

Each glandular hair has a **stalk** and **head-cell**, but the **head-cell alone is secretory**. The head-cell secretes between its cuticle and the remaining part of its cell-wall (the cellulose part) a **highly refractive resinous yellowish substance**. The cuticle is raised by the secretion, distended, and finally ruptured, whereupon the secretion often overflows the upper part of the hair.

Make out as many of the different types of hairs as you can. Remember that each hair is an outgrowth from a single epidermal cell.

Make a drawing showing (1) **ordinary epidermal cells** in cross-section as you have seen them in a transverse section of the petiole, (2) a **very long bristle**, (3) a **short bristle**, (4) a **long glandular hair**, (5) a **short glandular hair**, and (6) any other hairs that have interested you. In each glandular hair indicate the **stalk** and the **head-cell**. If you have seen the secretion in a head-cell lying between the **cuticle** and the rest of the **cell-wall**, indicate it in your drawing. All the drawings referred to above may be combined in a single semi-diagrammatic drawing of a single piece of epidermis. The long bristles should be made 3-4 inches long, and everything else should be drawn in proportion.

CHAPTER XV

CLOSTERIUM. COTTON WOOL AND A STUDY OF CELLULOSE

Materials and reagents supplied :

- (1) Closterium, in a drop of water, obtained with the help of a pipette from above the mud in an aquarium in the laboratory ;
- (2) Iodine, for testing for starch in the chloroplasts of Closterium ;
- (3) A Cotton fruit (to be passed around) ;
- (4) A Cotton seed covered with epidermal hairs ;
- (5) Cotton wool, dry, for a study of cellulose ;
- (6) Paper of various kinds : blotting-paper, filter paper, newsprint (*Free Press* paper), brown paper, writing paper, and lens paper, to be used for studying the chemical reactions of cellulose ;
- (7) Chlor-zinc iodine, to be used as a test for cellulose which it stains violet ;
- (8) Sulphuric acid, strong (2 parts H_2SO_4 , 1 part water), to be used in conjunction with iodine, as a test for cellulose (the membranes swell up and turn blue) ;
- (9) Ammoniacal cupric oxide, also called cuprammonia, to be used as a test for cellulose which it completely dissolves. Freshly prepared from copper sulphate by precipitating copper hydroxide by means of a weak solution of ammonium hydroxide, by well washing the precipitate, and by then dissolving the precipitate in strong ammonia.

A. CLOSTERIUM, A UNICELLULAR ALGA

Closterium is an Alga and therefore belongs to the same group of Flowerless Plants (Cryptogams) as **Protococcus** already studied. There are Green Algae, Blue-green Algae, Brown Algae (brown Sea-weeds), and Red Algae (red Sea-weeds). Closterium, like Protococcus, is one of the **Green Algae**. Included in the Green Algae is a family known as the **Desmidiaceae**, and it is to this family that Closterium belongs. We may therefore say that Closterium is a Cryptogam, a Green Alga, and a Desmid (or one of the Desmids).

The **Desmids**, of which there are many species, are remarkable for their great diversity of form and their wonderful symmetry, and some of them are among the most beautiful of microscopic objects. They are all unicellular, unattached to any substratum, and mostly free-floating, but some of them, including **Closterium**, move over the surface of mud, etc. They thrive best in soft water and are most numerous in peaty water with a trace of acidity. They frequently occur in enormous numbers in small ponds and in the quiet margins of lakes. **Closterium** is one of the best-known genera in the family.

Origin of the Material supplied.—In one of the laboratories of the Botanical Department, at a window, is a large glass tank or **aquarium** with a layer of sand and mud at the bottom and filled to a height of about 6 inches with water obtained from the Red River. In this aquarium there are many species of Green Algae constantly present and among them one, and often two, species of **Closterium**. On bright days one can observe **dark-green patches**, an inch or more wide, **on the mud**. Here there are thousands of **Closterium** plants and, in order to obtain them for class use, one sucks up the water in which they lie by means of a long pipette. The material to be used to-day was obtained in this way. Naturally, some of the mud comes up into the pipette along with the **Desmids**, and hence it is that in your preparation you will find the **Closterium** plants mixed up with bits of organic *débris*, etc.

To Make a Preparation of *Closterium* Plants.—The liquid which was pipetted up from the surface of the mud contains hundreds or thousands of **Closterium** plants and is now in a small beaker. **Place a clean slide on your table number**, and then a demonstrator, with the help of a pipette, will give you a drop of the **Closterium** fluid. Add a cover-glass and examine the preparation with the microscope, using the low-power objective first and then the high-power.

Observations with the Microscope.—Bring the preparation into focus with the **low-power objective**. With arms resting on the table and with the first fingers and the thumbs of both hands applied to the ends of the slide, move the slide about on the stage and **explore the preparation** thoroughly. See that you have **correct illumination** (with both hands on mirror, turn mirror until the strongest possible light comes up the tube, and then cut down the light with the iris diaphragm : by proceeding thus,

you will obtain the sharpest possible image of the object and see details to best advantage). Note the irregular masses of organic débris, and **look for Closterium plants**. These can be readily recognised as conspicuous bright-green organisms, much longer than they are broad, and pointed at each end. Notice their peculiar shape. How many placed end to end would be equal to the diameter of the field of view as seen with the low-power objective? How long is a Closterium plant?

Notice that each Closterium plant is **free from its substratum**, i.e. not attached in any way, and is **solitary**. Closterium differs from Protococcus in never forming colonies or aggregations.

Do any of your Closterium Plants exhibit Locomotion?—Closterium is able to move about slowly over its substratum, owing to the fact that it **excretes mucilage** at one end of its cell. The movement is a **sliding** one and usually rather slow and irregular. Some of your plants may be quiescent, but others may be moving slowly. The movement can be best observed with the **high-power objective**. The mucilage excreted behind a moving Closterium is invisible in such a preparation as you have, but it can be made visible by causing the organism to move among the particles of indian ink. The mucilage forms a sort of broad stalk which keeps growing in length, thus pushing the organism forward.

The Structure of a Closterium Plant.—A Closterium plant is elongate and strongly attenuated toward the poles, and it is never quite straight but more or less curved. Some species are lunate (half-moon shaped) or arcuate (bent like a bow), but the species now under observation is almost but not quite straight. Observe that **the long axis of each plant is slightly curved**.

Each Closterium plant obviously consists of a **single cell** and, therefore, like a Protococcus plant or a Yeast plant, is a **unicellular organism**.

If one were to cut a Closterium plant in two across its middle, the two halves would be exactly alike. The organism, therefore, is **bilaterally symmetrical**.

Each plant is invested by a thin firm cellulose **cell-wall** which can be readily seen, and on the outside of this is an invisible thin layer of gelatinous matter. **Look for the cell-wall**.

The living protoplasm consists of **cytoplasm**, a central **nucleus**, and **two chloroplasts** one in each half-cell (semi-cell).

The **cytoplasm** is largely hidden, owing to the green colour and

relatively large size of the two chloroplasts embedded in it. It can be detected on the sides of the organism, **just inside the cell-wall**, where it may exhibit the phenomenon of **protoplasmic streaming**; it can clearly be seen at the two ends (poles) of the cell, because the chloroplasts do not stretch so far; and there is a large mass of it in the centre of the cell between the two chloroplasts.

Study the cytoplasm at one of the ends of a cell. Notice that it is **colourless** and that it contains a globular **vacuole**, filled with **cell-sap**, in which are a number of tiny particles which exhibit a curious trembling movement. The particles are crystals of **gypsum** (calcium sulphate, CaSO_4). Gypsum is used for making plaster of Paris. The crystals (prisms and plates) are, of course, non-living, and their movement, known as **Brownian movement** (after Robert Brown, a distinguished English botanist), is due to uneven molecular bombardment by the molecules making up the cell-sap in the vacuole. All very minute free solid particles suspended in a fluid exhibit Brownian movement.

The **nucleus** is embedded in the cytoplasm in the middle of the cell, half-way between the poles. In a number of species of Closterium the two chloroplasts do not come together in the middle of the cell, so that there is a gap between them, and then there is no difficulty in seeing the cytoplasm there and also the nucleus it contains; but, in the species provided to-day, the **chloroplasts come up to the middle of the cell** where they touch or almost touch one another, and together they hide the nucleus from view. By focussing up and down in the middle of the cell, it is possible to see something of the central mass of cytoplasm which, although actually colourless, looks pale green owing to the fact that it is seen through parts of the green chloroplasts.

The **chloroplasts** are **two** in number, one in each half-cell. The chloroplast in a half-cell consists of a thick green, axial, rod-like mass, running down the centre of the half-cell, and from it there project radially outwards some six or eight green **longitudinal ridges** or flange-like plates. These longitudinal ridges of the chloroplasts can be readily seen when the upper surface of the organism has been brought into focus. By focussing up and down under the high-power objective, the number of ridges in view may be counted.

The central axial part of each chloroplast contains a series of

about eight or nine rounded **pyrenoid bodies** set in it at intervals along its axis. A pyrenoid body is a small colourless part of a chloroplast, made up of proteins, around which a **layer of starch grains** is formed during photosynthesis, and it is therefore to be regarded as an **active centre of starch formation**. Pyrenoid bodies are found only in Green Algae and in Diatoms. In addition to the starch grains surrounding each pyrenoid body there are numerous **very tiny starch grains** scattered **throughout the general mass of each chloroplast**, and they can readily be observed in the longitudinal ridges as greenish glistening particles, the colour being due to the chloroplast in which they are embedded. It is believed that these scattered starch grains are formed in the first place in the pyrenoid bodies and that they are then transported into the other parts of the chloroplasts. On this view, all the starch grains in a Closterium plant are of pyrenoid-body origin.

Make a drawing of a single Closterium plant about five inches or more long. As a **guide-line** (to be rubbed out later), draw a slightly curved line to mark the **central axis** of the organism, and then build up the outline of the cell symmetrically about it. Indicate the **cell-wall**, the **two polar vacuoles** with their **gypsum crystals**, the **cytoplasm** at the poles, etc., the **two chloroplasts** with their longitudinal ridges, the central row of **pyrenoid bodies** with their starch sheaths, the light central oval mass of cytoplasm which marks the **position of the nucleus**, and the minute **starch grains** scattered through the chloroplasts. As usual, write above your drawing what it is meant to represent.

The Action of Iodine on a Closterium Plant.—Place a drop of iodine at the edge of the cover-glass and let it diffuse slowly under the cover-glass until it reaches some of the Closterium plants. As soon as the iodine begins to act upon a Closterium plant, notice that the sheath or shell of **starch grains surrounding each pyrenoid body** quickly becomes **blue-black**. Notice, further, that the numerous **tiny starch grains** in the longitudinal ridges of the chloroplasts also turn **blue-black**. Notice, still further, that the **cytoplasm** turns **yellow** and often slightly **contracts** away from the wall at one end of the cell.

The observations just made with the help of iodine go to show that Closterium, even although it is a unicellular plant, resembles the multicellular Flowering Plants in being able to manufacture sugar from carbon dioxide and water with the aid of energy

derived from the sun's light, and in being able to convert some of this sugar into starch. There is every reason to suppose that photosynthesis runs a course which is essentially the same in all green plants.

It is interesting to compare and contrast **Protococcus** and **Closterium**. The two organisms resemble one another in being unicellular green plants, but they differ from one another in many ways, e.g. their **habitat** (the kind of place in which a plant grows), their general form, the number and complexity of their chloroplasts, their power of movement, and their vacuoles. It may be added that *Closterium* reproduces itself by cell-division and also by conjugation, but these processes cannot be described here.

B. THE EPIDERMAL HAIRS OF COTTON SEEDS

The cotton used in commerce is derived from the seeds of low shrubs of the genus *Gossypium* which, along with the Hollyhock and the Mallow, belongs to the natural family of Dicotyledons known as the **Malvaceae**.

Raw cotton (lint, cotton-wool) is simply a mass of very long unicellular hairs torn away from the testa of the seeds of the Cotton plant. In the United States, cotton is grown in ten of the southern States and ranks third in crop values (corn and wheat take first and second places). When the fruit (bolls) of the Cotton plant ripen, they open and expose numerous seeds, each one of which is enveloped and almost hidden by its own very numerous and elongated epidermal hairs. In the cotton fields of the southern United States the cotton seeds are picked out of the bolls by hand and later the epidermal hairs are separated from the seeds by means of a machine called the **cotton gin**. The Hindoos appear to have been the first to weave cloth out of cotton fibres, and for upwards of 3000 years (1500 B.C.—A.D. 1500) India was the centre of the cotton industry. When Pizarro, a Spanish discoverer, first came to Peru, in 1522, he found cotton in use there.

Cotton Fruits (Bolls).—A Cotton fruit which has opened and shows its seeds will be passed around to you for your inspection.

Examination of Cotton Seeds.—A Cotton seed with its epidermal hairs still attached is provided. Examine it carefully. Note, by feeling, the size of the seed. How long are the epidermal hairs? Are they stiff and straight, or flexible and irregularly waved?

Make a drawing of a Cotton seed, natural size, showing the epidermal cells coming away from the testa in all directions.

To Make a Microscopic Preparation of a Single Epidermal Hair.—Place a drop of water on the middle of a slide. With a finger and thumb pull away from a cotton seed **one** of its epidermal hairs. Lay it on the drop of water and add a cover-glass. Examine the preparation first with the high-power objective and then with the low.

Observations with the Microscope.—With the help of the **low-power** objective, note that the hair is very long relatively to its width. While its width is only about 0.01–0.03 mm., its length may be (as here) 10 mm., so that the hair is roughly about 500 times as long as it is wide. In so-called **long-staple** cotton the hairs may be 6 cm., or upwards of two inches, in length. Move the slide about on the stage and **observe every part of the hair** from one end to the other.

Notice that the hair is somewhat **flattened** and at the same time **twisted** about its own axis, and that it is **wider in the middle part than at either end**. **How many twists can you observe?** The twists are of the greatest importance for the cotton industry, for it is owing to their presence that it is possible to spin the raw cotton mechanically into the **yarn** which is used for making sewing thread, lace thread, stocking thread, and thread for weaving cotton cloth. If there were no twists in cotton fibre, it would be more difficult to make durable cotton goods out of it.

Observe that the **cell-walls** are decidedly **thick** (thicker than those of most epidermal hairs). The **cell-lumen** (cavity of the cell), since the hair is somewhat flattened, must be somewhat flattened too. While the hair was growing, it had the usual cell-contents (cytoplasm, nucleus, vacuole filled with cell-sap); but, when the hair attained its full length, it **died**; its protoplasm disappeared, its cell-sap evaporated, and **its lumen became filled with air**. In your hair, most probably, the air has been mostly displaced by water; but here and there you may find elongated air-bubbles which, if present, you will easily recognise by their peculiar dark appearance.

Cotton Consists Chiefly of Cellulose.—A chemical analysis of cotton was found to be as follows: **cellulose** 91 per cent., **water** 8 per cent., with traces of wax, fat, oil, protoplasm and its derivatives, and mineral matter (salts of K, Na, Ca, Mg, Fe, and Al).

The cellulose makes up the cell-wall and was formed in successive layers by the living protoplasm of the cell.

Make Two Drawings.—(1) The whole of a single hair slightly magnified. Make the hair about 6 inches long. Draw it with a single line. (2) Part of a hair highly magnified. In this drawing the hair should be made about half an inch wide at its widest part. Represent a few **twists** and show the exact thickness of the **cell-wall** relatively to the breadth of the **lumen**. Indicate what your drawings are meant to represent.

C. REMARKS ON CELLULOSE

Cellulose.—The term **cellulose** is applied to a group of substances isolated by the cytoplasm of the living cell to form the **cell-wall**. Cellulose is manufactured by all the Flowering Plants (Phanerogams) and by nearly all of the Flowerless Plants (Cryptogams). Among the exceptional plants which do not have any cellulose in their cell-walls are most Fungi and many Bacteria. **In the Animal Kingdom**, on the other hand, **cellulose is almost universally absent**. There is no cellulose in any of the Vertebrata, and among the **Invertebrata** it has only been found in certain cells of certain Ascidians (Sea-squirts) and Insects. The presence or absence of cellulose, therefore, is a general test of considerable importance in distinguishing between plants and animals.

Chemically, cellulose, like starch and sugar, is a **carbohydrate**, i.e. a chemical compound containing **carbon, hydrogen, and oxygen**, in the molecule of which there are two hydrogen atoms to each oxygen atom. Its molecule is large and complex and has the formula $(C_6H_{10}O_5)_n$.

Cellulose is **hard** and **colourless**. It **absorbs water** readily and yet is **insoluble in water**. On account of its possessing these properties, cellulose is peculiarly well-fitted to be used as plants use it, namely, as material for the construction of cell-walls. Its lack of colour and its transparency enable light to pass through it easily and penetrate into leaves, etc., during photosynthesis. Its hardness and strength give a cell-wall firmness and power to resist crushing and tensile strains. Its property of absorbing water (by **imbibition**) and of allowing water to pass through it easily permits of water (one of the chief constituents of a plant, over 50 per cent. by weight of a living plant is water) entering a plant and passing without difficulty from cell to cell.

It has been possible to take a cotton hair, measure its cross-sectional area, suspend the hair, hang on to it weights, and find the weight which will just cause it to break, and thus to determine the tensile strength of the hair. In one instance this was found to be 4·2 tonnes per square centimetre, or about 25 English tons per square inch. This tensile strength is about the same as that of wrought iron. Cellulose membranes are **not only very strong but they are wonderfully elastic**. Ripe wheat or rye, etc., growing in a field, bends and bows beneath each blast of wind; but, as soon as the blast has gone, all the slender stems, laden though they are with heavy heads of grain, return again to their original upright positions. This yielding momentarily to the wind and recovery of position when the wind has passed is solely due to the properties of moist cellulose.

There are three chief types of cellulose :

- (1) **Typical cellulose** of the Cotton Type (cotton, flax, hemp, etc.) ;
- (2) **Compound celluloses** of the Wood, Jute, and Cereal Grass Types ; and
- (3) **Hemi-cellulose** or **Reserve cellulose** of the cell-walls of the seeds of Coffee, Date, Lupin, etc.

(1) **Typical cellulose**, e.g. that of cotton, flax, and hemp, is almost pure cellulose and reacts to the tests for pure cellulose which are to be tried shortly.

(2) The **compound celluloses** in wood, jute, and grass fibres consist of cellulose united with some form of non-cellulose compounds. These celluloses do not take on the reactions for pure celluloses. The cell-walls of wood cells are said to be **lignified** or to consist of **ligno-cellulose**, and such membranes can be identified by microchemical tests for "**lignin**."

(3) **Hemi-cellulose**, which we have seen in the walls of the endosperm of Coffee and Date seeds, is a non-fibrous cellulose and is very readily converted into certain sugars (mannose, galactose and pentose) when the seeds are germinating and the embryo requires a supply of nutriment. Hemi-cellulose gives reactions different from those given by Typical cellulose.

Cellulose is used industrially for many important purposes. **Cotton** and **flax** fibres are used for making cotton and linen goods respectively ; while **hemp** fibres are used for making canvas, tent-cloth, sail-cloth, bags, sacks, cordage, and ropes. While

cotton is derived from the epidermal hairs of seeds, flax and hemp fibres are derived from bast fibres contained in the vascular bundles of stems.

Paper is chiefly made from cellulose obtained from Spruce wood. In inferior quality papers, e.g. newsprint, the encrusting lignin is removed only from part of the cellulose (from the chemical pulp, not from the mechanical); but, in high quality paper, e.g. writing paper, the lignin is removed by boiling with calcium bisulphite, a fairly pure form of cellulose (sulphite cellulose) being thus produced.

Cellulose used for the preparation of **filter paper** (made from the best qualities of cotton rags) is first purified by the sulphite method and it is then treated with hydrofluoric acid to remove any silica which may be present. **Filter paper consists practically of pure cellulose** and therefore gives the reactions for pure cellulose very strongly. The purity of the filter paper is a matter of considerable importance for filtering operations in chemical laboratories.

Among the commercial substances in which cellulose is employed are: **gun cotton** (used for making smokeless powder), **blasting gelatine** (used in cordite, etc.), **collodion**, **celluloid**, **viscose**, and **artificial silk**. Artificial silk, or **rayon** as it is now often called, is made in several different ways, one of which is by dissolving cellulose (of the walls of the tracheides of Spruce wood, etc.) by means of cuprammonia and then running a fine stream of this solution into dilute sulphuric acid, the result being that a fine continuous **thread of cellulose** is formed by precipitation. The artificial silk industry, which is now highly developed, is therefore essentially a cellulose industry.

Cotton can be made to look very much like silk by subjecting it to a process known as **mercerising**. This process was discovered by John Mercer in 1850. The cotton fibre is soaked for an hour in a concentrated (33 per cent.) solution of caustic soda (NaHO). The fibres so treated become thicker, more cylindrical, and more translucent, and later they can be dyed to better advantage. The finished goods, known in the trade as **mercerised cotton**, are often brilliantly coloured and always have a gloss (sheen) resembling that of silk.

D. MICROCHEMICAL TESTS FOR CELLULOSE

I. First Series of Tests. To be made with Cotton Wool.

Handle the chemical reagents **carefully**, and do not get spots of them on the table, the microscope, or on the upper surface of the cover-glass. The **low-power** objective **alone** is required for observing the reactions. If you put on the high-power, be very careful that it does not come into contact with any of the reagents. Remember that **strong sulphuric acid** is a **particularly dangerous** chemical compound that burns holes in clothes and corrodes metals, etc.

The first two tests, for convenience in making comparisons, should be made in succession side by side on one and the same slide.

(1) **Add iodine.** Pull out from the dry cotton wool provided a thin fluffy bit (20–50 fibres or so), place it on a **dry slide** and then add a cover-glass. **No mounting fluid is to be used.** Now run under the cover-glass in the usual way a drop of **iodine**. Within a minute the iodine will have coloured the fibres. Look at the preparation **macroscopically** against a background of white paper and then **microscopically** under the low-power objective. Have the fibres become yellow or brownish-yellow or brown? **Record the reaction** in your book.

(2) **Add chlor-zinc iodine.** As before, pull out from the dry cotton wool provided a thin fluffy bit, place it on a **dry slide** (the slide just used will do well), and then add a cover-glass. Again, **no mounting fluid** is to be used. Now run under the cover-glass a drop of **chlor-zinc iodine**. Within a minute the reagent will have begun to turn the fibres **violet** and, in course of time, the violet will become **dark violet**. The zinc chloride has altered the cellulose in some way, so that the iodine present in the solution now turns the cellulose violet. Look at the preparation **macroscopically** against a background of white paper, and **microscopically** under the low-power of the microscope. Compare the fibres treated with iodine alone with those treated with chlor-zinc iodine. Chlor-zinc iodine is the simplest reagent for detecting cellulose by means of a characteristic reaction. **Record the reaction** in your book.

(3) **Iodine followed by strong sulphuric acid.** As before, pull out from the dry cotton wool provided a thin fluffy bit, place it

on a dry slide. Do **not** put on a cover-glass, but add to the wool **a drop of iodine**. With a needle press the fibres into the iodine. As soon as the fibres have become brown to the naked eye, with the help of blotting-paper **suck away the liquid iodine** from the fibres. Then add a cover-glass. Then, exercising due care, run a drop of **strong sulphuric acid** under the cover-glass. As soon as the sulphuric acid comes into contact with the cotton fibres, it causes them to **swell up** and turn a **bright blue**. Verify this reaction macroscopically and microscopically and **record it** in your book.

(4) **Add ammoniacal cupric oxide**. This reagent is often called, for short, **cuprammonia**. As for tests (1) and (2), pull out from the dry cotton wool provided a thin fluffy bit, place it on a **dry slide** and add a cover-glass. **No mounting fluid** is to be used. Now run under the cover-glass a drop or two of **ammoniacal cupric oxide**, and, **immediately thereafter**, examine the preparation under the low-power of the microscope.

You should have no difficulty in observing the reaction. The cotton fibres swell up enormously and those which have their cellulose in the purest form become **entirely dissolved**. Others become partially dissolved; with longer action of the reagent and more of it, they might disappear altogether. Each hair appears to be covered over with a very thin **cuticle** and this to a considerable extent resists the action of the reagent. The wall of each hair is made up of successive layers of cellulose which were added one by one by the cytoplasm, as the cell-wall thickened. As the wall swells, it often shows some of these cell-wall layers. Try to observe them. **Record the reaction** in your book.

II. Second Series of Tests. To be made with Paper.

Now that you have become acquainted with the tests for cellulose, you may determine for yourselves whether or not cellulose is present in paper of various kinds.

(1) Take a piece of **white blotting-paper**. Using the pipettes in the reagent bottles, add a spot of **iodine** in one place and a spot of **chlor-zinc iodine** in another. Judging by the reactions as seen with the naked eye, is or is not cellulose present in the paper?

(2) Take pieces of the following kinds of paper: (a) **filter paper**, (b) **newsprint** used by the *Free Press*, (c) **brown paper**, and (d) **writing paper**. With the aid of the pipettes place thin streaks

of **iodine** and of **chlor-zinc iodine** on each kind of paper. Is cellulose present in these kinds of paper or not ?

Take a piece of the **newspaper** treated with chlor-zinc iodine, place it on a slide, and look at it with the low-power of the microscope. The long fibres stained violet are tracheides of Spruce wood freed from lignin and now practically pure cellulose. They were part of the **chemical pulp**. Between these long intact tracheides are numerous bits of what was **mechanical pulp**, i.e. wood ground up in little bits but not freed from lignin chemically.

(3) Take **three** small pieces of **lens paper**, each about one-eighth of a square inch in area, and place them dry under **three separate cover-glasses** on one slide. To the first preparation add **iodine**, to a second **chlor-zinc iodine**, and to the third **cuprammonia**. You should be able to observe that the fibres of the lens paper give the characteristic reactions for cellulose.

(4) Take another small piece of **lens paper**. Lay it flat on a dry slide. Do **not** add a cover-glass. Add a drop of **iodine**, sufficient just to stain all the fibres brown. **Suck away any liquid iodine**. Then add a cover-glass. Then run **sulphuric acid** under the cover-glass. Again you should be able to observe the characteristic reaction for cellulose (here a swelling of the fibres and a bright blue colour).

Record in your book whether or not cellulose is present in paper of various kinds and the nature of the evidence upon which you have relied for your conclusion.

CHAPTER XVI

STOMATA. ROOT-HAIRS. RAPHIDES. THE INTERNAL STRUCTURE OF THE STEM OF A MONOCOTYLEDON

Materials and preparations supplied :

- (1) A leaf of the Spiderwort (Wandering Jew) ;
- (2) A leaf of a Narcissus or of a Hyacinth ;
- (3) A leaf of the Canadian Pondweed ;
- (4) A Mustard seedling, with root-hairs on the primary root ;
- (5) Some fronds of the Ivy-leaved Duckweed (*Lemna trisulca*) ;
- (6) A stem of a Palm, to be passed around ;

- (7) A piece of a stem of Corn (*Zea mays*), pickled in alcohol ;
- (8) A transverse section of a Corn stem, cut with the microtome and stained ;
- (9) A macerated piece of Corn stem.

A. POSITION OF STOMATA ON LEAVES

Place each of the following leaves on the stage of your microscope and determine with the low-power objective **where the stomata are situated** :

(1) A leaf of the **Spiderwort**, also known as the **Wandering Jew** (*Tradescantia fluminensis*). Under natural conditions the lamina of this leaf is spread out in a more or less **horizontal** plane. Its lower (dorsal) surface differs in appearance from its upper (ventral) surface, and it can be described as a **dorsi-ventral bilateral organ**.

(2) A **Narcissus** leaf (or a **Hyacinth** leaf). This leaf has no petiole and is more or less **upright**. Its two sides look very much alike, and it can be described as an **isobilateral organ**.

(3) A leaf of the **Canadian Pondweed** (*Elodea canadensis*). This plant is a submerged aquatic, its leaves being entirely **immersed in water**. Put the leaf on a slide. No cover-glass is required.

Record your findings in your book.

B. ROOT-HAIRS OF A MUSTARD SEEDLING

The Mustard seedling provided came from a seed sown on wet blotting-paper in a covered crystallising dish some five days ago. A Mustard seed contains no endosperm and consists solely of a **testa** and an **embryo**. The embryo has a **radicle** and **two cotyledons**, but its plumule (between the two cotyledons) is very rudimentary or absent. The reserve food materials are stored in the cotyledons. In the seedling supplied the radicle has become the **primary root** and the two cotyledons are being raised up into the air by the stem (**hypocotyl**) to become the first two **green leaves**.

Macroscopic Observations on the Root and Root-hairs.—Notice with the naked eye that the root may be divided into : (1) a **root-hair region**, covered with long root-hairs ; (2) a **region devoid of root-hairs**, where elongation of the root and stretching in length of all the cells are taking place ; and (3) the **root-cap**.

The root-cap envelops and protects the internal **apical meristem** where new cells are coming into existence by cell-division.

To Make a Microscopic Preparation showing Root-hairs.—Put some water on a slide and lay the root in it (cut the root away from the stem or hypocotyl, if necessary). Add a cover-glass. The cover-glass will be raised up by the root, and it may be necessary to add two or three more drops of water so as to cover the root-hairs. Examine with the **low-power** objective.

Observations with the Microscope.—Notice the numerous, irregularly curved root-hairs on the older part of the root. These have attained their full length. New hairs, easily distinguished by their shortness, are growing out from single epidermal cells in acropetal succession, i.e. in succession toward the root-apex. Notice that each hair is **unicellular, thin-walled,** and **very long** relatively to its width, so that for its mass it has a **very large absorptive surface.** Root-hairs, unlike the hairs on leaves and stems, are **not covered by a cuticle,** so that water from the soil can readily be drawn into their interior.

With the **high-power** objective, owing to the thickness of the root, it is only possible to examine those root-hairs which may happen to lie **just under the cover-glass.** If a root-hair should be examined in this way, it will be found to contain cytoplasm and vacuoles. Its nucleus is usually near the apex.

Make a drawing of **two older root-hairs** and of **some younger** partially grown hairs. The long hairs should be drawn at least six inches long, and care should be taken to make the relative width as it should be.

C. RAPHIDES OF THE IVY-LEAVED DUCKWEED

Lemna trisulca, one of the Duckweeds, called in England the **Ivy-leaved Duckweed**, is a small submerged aquatic Flowering Plant, but its minute flowers are very rarely seen. It is found in still waters of lakes, ponds, etc. Each of its fronds (? stems) is oblong to oblong-lanceolate, 6–10 mm. long, attenuated at its base into a slender stalk, and **often without any rootlets at all.** Each frond may branch laterally, the two branches growing out at right angles from its two sides, so that the new fronds lie cross-wise to the old. It is believed that all the Duckweeds (**Lemnaceae**) have come, during the course of evolution, from large complex Flowering Plants like the Arum Lily (Trumpet

Lily). They have taken to the water and have lost their leaves and almost lost their roots (some species are always rootless), while their plant-body has become reduced to a mere frond which only occasionally bears minute flowers. The plants are multiplied by the fronds breaking apart.

To Make a Microscopic Preparation to show Raphides.—Take a single frond. Mount it whole in a drop of water on a slide, and cover with a cover-glass.

Observations with the Microscope.—With the low-power objective, and afterwards with the high, observe **toward the edge** of a frond : (1) the **green parenchymatous cells** containing chloroplasts ; and (2) **crystal sacs**, special more elongated cells, known by their shape, their freedom from chloroplasts (hence their whitish appearance), and their peculiar cell-contents. Each crystal sac contains a **bundle of raphides**, i.e. a bundle of very numerous **needle-like crystals of calcium oxalate**. These crystals may be considered as a **waste product** as, when once formed, they do not as a rule again enter into the plant's metabolism. Water snails do not eat the plant, and this may be due in part to the presence of the raphides. Possibly, therefore, the needle-like crystals have a protective function.

Make two drawings : (1) a **frond**, natural size ; and (2) a **crystal sac** containing a **bundle of raphides**. The sac should be drawn 2-3 inches long, and the surrounding, shorter, chloroplast-containing, parenchymous cells should be indicated.

To Tease out Raphides from the Crystal Sacs.—Remove the cover-glass from the preparation. Take two needles, or a needle and knife, and tear the frond into pieces in the drop of water. Some of the crystal sacs will break open during this operation and their raphides will be scattered in the water. After teasing, lay the cover-glass on the preparation again, add more water if necessary, and **look for isolated raphides**. They may be observed with the low-power objective, if the light is properly adjusted by partially closing the diaphragm and if one focusses on the upper surface of the glass slide (the crystals sink to this position by their own weight). The crystals will appear as **very minute needle-shaped structures**. When you have found some of them, examine them with the high-power objective.

Make a drawing of some isolated raphides. They should be drawn about two inches long.

D. THE INTERNAL STRUCTURE OF THE STEM OF A
MONOCOTYLEDON

Monocotyledons and Dicotyledons.—The Flowering Plants, other than the Pines and their allies, are divided into two great groups, **Monocotyledons and Dicotyledons**, which differ from one another in a number of important particulars.

In **Monocotyledons**, represented by Palms, Dragon trees, Bamboos and other grasses, Sedges, Rushes, Lilies and their allies, and Orchids : (1) the embryo has only **one** cotyledon ; (2) the leaves have **parallel** veins ; (3) the parts of the flowers are typically in **threes** ; and (4) the **vascular bundles** in the stem are **numerous and scattered**, and they are **not provided with a cambium**, so that the stem as a whole, when once formed, cannot increase in thickness. The stem has **no pith**, **no medullary rays** and **no annual (yearly) rings of wood**. If one looks at a cross-section of a Palm trunk, one cannot estimate its age owing to the absence of any annual rings.

In **Dicotyledons**, represented by Beans, Peas, Roses, Sun-flowers, Poplars, Elms, Basswoods, Oaks, and Birches : (1) the embryo has **two** cotyledons ; (2) the leaves are **net-veined** ; (3) the parts of the flowers are typically in **fives** or **fours** ; and (4) the **vascular bundles** in the stem are **few** in number, not scattered but **arranged in a ring**, and they are **provided with a cambium**, so that the stem as a whole, after its first formation, can grow in thickness. The stem has a central **pith**, **medullary rays** and, if it is a perennial (shrubs and trees), **annual rings of wood**. If one looks at a cross-section of the trunk of an Elm or an Oak, one can estimate the age of the tree by counting the annual rings of wood.

Corn (*Zea mays*).—To illustrate the structure of a typical monocotyledonous stem Corn will be taken. Corn is a grass and, along with Wheat and all wild grasses, belongs to the great natural family **Gramineae**. Most grasses have a hollow stem, but Corn is exceptional, for its stem is **solid**.

Macroscopic Study of the Stem of Corn.—With the naked eye examine the piece of an internode of a Corn stem provided. Notice how smooth and hard the **epidermis** is, and that through it one can perceive some of the **vascular bundles** as thin whitish strands of tissue running longitudinally down the stem. In the exposed **transverse** surface of the stem notice the numerous

vascular bundles embedded in the **ground tissue** (**fundamental tissue**).

With a sharp pen-knife or a scalpel cut the piece of stem vertically down the middle into two pieces. Examine the new surfaces thus exposed to view and observe the vascular bundles running longitudinally downwards in the ground tissue.

Cut **one** of the semi-cylinders vertically downwards into two equal pieces, and **draw one of these pieces** so as to show its upper surface and one of its sides. Indicate the **vascular bundles** and the **ground tissue**.

To Make a Thin Transverse Section of the Corn Stem.—Take the semi-cylinder (half-stem) already prepared, hold it upright in the left hand and from it, with the razor-blade held horizontally in the right hand, cut a series of thin fairly large transverse sections. As a preliminary to this operation, dip the material in water and put a drop of water on the razor-blade with a finger of the left hand. Without this **lubrication** the sections will not readily slide on to the razor. Try to make the sections as **transverse** as possible, as **oblique sections do not give good results** when seen with the microscope. Before beginning to cut a section, it is best to lay the razor a little way across the top of the piece of stem; and, in cutting, it is well to begin getting the section by starting at the hard outer tissues of the piece of material. Mount one or two of the best sections on a slide in a drop of water, and cover with a cover-glass. Examine the preparation with the microscope.

Observations with the Low-power Objective.—Move the preparation about on the stage of the microscope and explore it well.

Observe that the **epidermis**, as in leaves, consists of a single layer of cells which bounds the stem on its exterior.

Next find the **cortex**. This lies between the epidermis and the vascular bundles and consists of only about five or six layers of cells, its **outer part** being made up of about two layers of smaller cells with thickened walls and its **inner part** of about two or three layers of larger cells with thinner walls. The vascular bundles never intrude into the cortex except at a node where they pass through it on the way to a leaf. In many Monocotyledons the cortex is sharply marked off from the central cylinder, but this is not so in *Zea mays*.

The **central cylinder** or **stele** is all the tissues internal to the cortex, i.e. most of the stem. The central cylinder consists of

nothing but numerous **vascular bundles** and of the **ground tissue** in which they are embedded. Notice that the vascular bundles toward the exterior of the central cylinder are more numerous and closer together, while toward the centre of the cylinder they are fewer and more widely separated. Notice also that there is **no pith** in the centre of the stem.

Observe that **each vascular bundle** is more or less **wedge-shaped** with the point of the wedge directed toward the centre of the stem, and that the **xylem** (wood), known by its two large **pitted ducts** and its smaller **spiral** and **annular vessels**, is situated on the **inner** side of each bundle (the side toward the centre of the stem), whereas the **phloem** (bast), known by its alternating **sieve-tubes** and **companion cells**, is situated on the **outer** side of each bundle (the side toward the epidermis).

It will be seen that in each vascular bundle the most conspicuous elements are the two great **pitted ducts**. These are hollow pipes which have lost their protoplasm. They extend from the root-tips through the stem into the leaves; and, in the mature stem, it is through them that water with salts in solution ascends to the leaves (ascent of sap).

Observe that the **ground tissue** in which the vascular bundles are embedded consists of **parenchymatous cells**, i.e. of cells which are **living**, **thin-walled**, and **isodiametric** (about equal in diameter in all directions). Between these cells, wherever three meet, there are **intercellular spaces** which can readily be seen. In each cell there is a thin primordial utricle (cytoplasm) pressed against the cell-wall by the cell-sap (when the cell is living), and the cell-sap contains salts and sugar in solution. In the **Sugar-cane** there is so much sugar stored up in those cells that it is expressed for commercial purposes. Notice that many of the ground-tissue cells are as large as, or even larger than, the two pitted ducts in each vascular bundle.

Before going farther, it may be advantageous to survey a list of all the tissues and cell-structures which are present in a cross-section of *Zea mays*.

An Analysis of the Tissue in a Corn Stem.

- A. **Epidermis**—one layer of cells thick, occasionally stomata may be seen in it.
- B. **Cortex**—

{	Outer , 2-3 layers of thick-walled smaller cells.
	Inner , 2-3 layers of thin-walled larger cells.

C. Central Cylinder (Stele) :

I. Ground Tissue—thin-walled living parenchymatous cells.

II. Vascular Bundles :

- | | |
|-----------------------------|--|
| (1) Phloem (bast) | { Sieve-tubes.
Companion cells. |
| | { Two large pitted ducts.
One spiral vessel.
One annular vessel (some-
times two). |
| (2) Xylem (wood) | { Xylem parenchyma—smaller
more numerous cells with
more or less lignified cell-
walls.
An air-space around the inner
side of the annular vessel. |
| (3) Bundle Sheath | { Thick-walled, narrow, scleren-
chymatous cells surround-
ing the phloem and xylem. |

Observations with the High-power Objective.—Examine the section from without inwards. Notice that the **epidermis** consists of a single layer of cells and that the cells are rather small and have thick walls. The walls are very hard and this is doubtless due to the cellulose being impregnated with **silica** (SiO_2 , the chemical compound which makes up quartz sand particles, it is harder than steel), as such an impregnation of the epidermal cell-walls is the rule in the stems of grasses (it occurs in Wheat straw, for example).

The **cortex**, not sharply marked off from the central cylinder, lies just inside the epidermis, never contains any vascular bundles, and is not more than about 5–6 layers thick. It may be divided into an **outer** and an **inner** cortex. The outer part, made up of 2–3 cell-layers, consists of small cells with thick walls, much resembling the cells of the epidermis. Along with the epidermis the outer cortex gives mechanical rigidity to the exterior of the stem. The **inner** part of the cortex, made up of about 2–3 cell-layers, consists of larger cells with thinner walls. These cells, when alive, contain green chlorophyll in their chloroplasts, but in your material the chlorophyll has been extracted by the alcohol which was used as a preservative.

The **central cylinder** may now be examined. It occupies the

whole of the remaining part of the stem. As already remarked, it consists solely of **vascular bundles** and **ground tissue**.

Examine a vascular bundle in detail. It is best to choose one about half-way between the centre and the circumference of the stem. With the low-power objective, survey the section and find the best vascular bundle for further observation. Then **turn the slide** until the two great pitted ducts are to the right and left of the field of view, the rest of the xylem is turned toward you, and the phloem is directed away from you. The vascular bundle is best studied and sketched when it has this orientation. Turn on the high-power objective.

In studying the bundle with the high-power objective, notice first the two great **pitted ducts**, which are either rounded or slightly oval in form. They are devoid of protoplasmic contents. Between the pitted ducts and slightly toward the centre of the stem (toward you) is the **spiral vessel**, while next to it and still farther toward the centre of the stem is the **annular vessel**. The spiral vessel has a spiral thickening inside it and the annular vessel annular (ring-like) thickenings. Something of these thickenings may be perceived. Below (toward you) and around the annular vessel you will observe a smaller or larger **air-space**. This air-space was formed by rupture of the tissues as the stem was thickening and elongating. In cutting the section it sometimes happens that the ring-like thickenings (annuli) of the annular vessel become broken away from the rest of the wall (which is relatively thin) and then they may be seen lying in the air-space tilted at various angles.

The remaining xylem cells are small fairly thick-walled **parenchymatous cells** with their walls more or less lignified. These cells fill up the space between the pitted ducts and around the spiral vessel, and they doubtless serve to strengthen the xylem mechanically.

It may here be stated that **the walls of xylem (wood) cells consist**, as a rule, not of pure cellulose but of **ligno-cellulose**. The cellulose is encrusted with substances including **lignin**, **wood-gum**, and **hadromal**. The walls of wood cells are said to be **lignified**. They do not take the microchemical reactions of pure cellulose. They do not turn violet with chlor-zinc iodine, or blue with iodine and sulphuric acid, and they are not dissolved by ammoniacal cupric oxide. But they have peculiar reactions of their own. For instance, they turn **crimson red** with phloroglucin and hydro-

chloric acid, and **yellow** with aniline sulphate and sulphuric acid ; whereas pure cellulose, when treated with these reagents, remains colourless. The lignification of wood cell-walls hardens them and renders them more permeable to water, thus rendering them more fit to carry out their functions. **In staining sections** of the stem of Corn, **two kinds of dyes are often used** ; one stains all the lignified membranes (pitted ducts, spiral and annular vessels, bundle-sheath cells) while the other stains only the membranes made of pure cellulose (phloem cells, ground tissue cells). Such differential staining may be seen in some of the prepared sections supplied to you.

The **phloem** (bast) can readily be made out, as it consists solely of **sieve-tubes** and **companion cells** which alternate with one another so as to give a more or less regular pattern. A **sieve-tube** is considerably larger than a companion cell, appears to be devoid of contents (under natural conditions it has contents of course), tends to be octagonal in shape (8-sided), and is usually surrounded by four companion cells. **The companion cells** are easily distinguished by their smaller size, by having 4 sides and by being filled with a dense but more or less contracted mass of protoplasm. Thus, while the sieve-tubes appear to be empty, the companion cells are seen to have abundant cell-contents. One companion cell is cut off from each sieve-tube when the phloem is being formed. Notice that the phloem cells have very thin and delicate cell-walls.

The details of structure of the xylem and phloem having been made out, the only other structure connected with the vascular bundle which remains to be examined is the **bundle sheath**. This sheath surrounds each bundle, but it is usually better developed (i.e. is wider and consists of more cells) on the **outer** and **inner** sides of the bundles than on their lateral sides. The bundle-sheath cells are somewhat narrow in cross-section but they are much elongated in the longitudinal direction of the stem. They have thick lignified cell-walls and are closely set together, so that they give mechanical strength to the vascular bundle as a whole.

The **ground tissue cells**, as may readily be observed, are of relatively large size. They have thin walls and large cell-lumina. Notice the **intercellular spaces** where the cells meet in threes. In each cell-lumen there is a primordial utricle in which a nucleus is embedded. The **nucleus** with its **nucleolus** may often be distinguished.

Make a drawing showing the main features of a single vascular bundle, and as many details as time permits. The drawing should be made the full width of the page. **Put in first the two large pitted ducts**, right and left, and make each duct about 1-1.25 inches in diameter. Then add the **spiral** and **annular** vessels and the **air-space** ; then the **wood parenchyma** ; then the **phloem (sieve-tubes and companion cells** ; then the **bundle-sheath** ; and, finally, some of the surrounding **ground-tissue cells**. As usual, indicate what your drawing is meant to represent and the names of the various parts.

Material for Observing the Cell-elements of a Corn Stem in Longitudinal View.—Some pieces of a corn stem have been **macerated** by heating them gently in potassium chlorate dissolved in nitric acid (Schultze's maceration fluid), with the result that the **middle lamella** between adjacent cells has been **dissolved away**, so that **the cells can now be separated from one another with the greatest ease**. The maceration fluid has also removed the encrusting substances from the cellulose in the cell-walls, so that these are no longer lignified but consist practically of pure cellulose.

To make a Preparation of Macerated Corn-stem Tissue.—Take the piece of macerated stem provided, with your razor or a sharp scalpel cut away from it a small piece about **one-eighth of an inch long**, lay the piece on a drop of water on a slide, and then **tease** it out with two needles or a needle and knife until its cell-elements are well separated from one another. Then add a cover-glass and examine the preparation first with the low-power objective and then with the high.

Observations with the Microscope.—Find (1) a **pitted duct**. This can be distinguished by the numerous slit-like **pits** on its lateral walls (hence the name **pitted duct**). Notice that the duct is made up of successive cells which have become fused together. Each cell has more or less oblique ends. Between any two of the cells one can observe the remains of a **septum** (cross-wall) which once separated the lumina of the two cells from one another but was finally dissolved away by an enzyme (**cytase**) until it was reduced to a mere **rim**. Also find (2) an **annular vessel**, known by its curious ring-like or annular thickenings, and (3) a **spiral vessel**, with its spiral thickenings. Other elements which should be found are : (4) **bundle-sheath cells**, known by their narrow elongated form, their thick walls, and their pointed ends ; and (5) **ground-tissue cells**, known by their considerable width, their

shortness in the longitudinal direction, and their thin walls. The **phloem** is not likely to be found. It is so delicate that its walls may have disintegrated during the maceration process.

Make drawings of : (1) a **pitted duct**, (2) an **annular vessel**, (3) a **spiral vessel**, (4) a **bundle-sheath cell**, and (5) some **ground-tissue cells**. Use your own judgment as to scale. As usual, indicate what your drawing is meant to represent.

CHAPTER XVII

BACTERIA. THE INTERNAL STRUCTURE OF THE STEM OF A DICOTYLEDON

Materials and preparations supplied :

- (1) Bacteria in a hay infusion kept at room temperature for 36 hours ;
- (2) Spirilla in water containing some dead snails, the water inoculated with bacteria in mud taken from an aquarium, kept at room temperature for about 7 days (this culture to be used only if Spirilla are abundant) ;
- (3) Bacteria in water containing some crushed peas, kept at room temperature for 36 hours ;
- (4) Two transverse sections of a young stem of the Castor Oil plant (*Ricinus communis*) in which a cambium ring has developed : Section A, cut by hand ; Section B, cut by the microtome ;
- (5) Transverse section of a twig of a Basswood tree (*Tilia europaea*), some five years old, to show secondary xylem (annual rings of wood) and secondary phloem (bast) produced by the cambium.

A. BACTERIA

Bacteria, often referred to popularly as **germs** or **microbes**, are the smallest known living organisms. They are a dominant group of plants, made up of numerous species. Vast numbers of bacteria surround us everywhere. Bacteria are present in the air we breathe, the water we drink, and in the earth on which we tread. They become attached to our skin and they invade our

bodies. Many species reside in our mouths and others live continuously in our alimentary canals. When a child is born, it quickly comes into contact with bacteria; and from childhood to youth, from youth to old age, bacteria are our constant associates. Finally, bacteria bring about the dissolution of our bodies and are the agents which return "dust to dust," thus destroying the dead and making the world once more sweet and wholesome for the living.

Bacteria are so excessively minute that they cannot be seen with the naked eye. We may therefore refer to them as **micro-organisms**, i.e. organisms which can only be seen with the microscope. We have already studied certain other micro-organisms, e.g. **Protococcus** and **Saccharomyces** (Yeast), but bacteria are much smaller than these. Some bacteria are so small that it would take twenty of them placed end to end to go across a yeast plant. The unit of measurement for measuring the size of bacteria is one micron (1μ) = one-thousandth of a mm. (0.001 mm.) = one-twenty-five-thousandth of an inch (0.00004 inch). Some of the spherical bacteria we shall see to-day are about one micron in diameter, while some of the rod-shaped bacteria are about one micron wide and several microns long.

Bacteria, like **Protococcus**, **Saccharomyces**, and **Closterium**, each consist of a single cell and are therefore **unicellular organisms**. They differ from **Algae** and they resemble **Fungi** in that they have **no chloroplasts** and **no chlorophyll**. They therefore cannot carry on the process of photosynthesis, i.e. they cannot synthesise sugar from carbon dioxide and water with the help of energy derived from the light of the sun. Instead of living an independent life like the green plants, bacteria depend on organic matter which has been built up by other organisms. When they feed on **dead** organic matter they are known as **saprophytes**, when on **living** organic matter they are known as **parasites**. The number of parasitic bacteria is comparatively few. Most bacteria are saprophytes and perfectly harmless to both animals and plants. Bacteria have been called **our secret friends and foes**. Among our secret friends are the bacteria of putrefaction which destroy dead bodies, the bacteria which fix free nitrogen in the soil, the bacteria which help to ripen cheese, to ferment tobacco leaves, and to make vinegar. Among our secret foes are those bacteria which are parasitic on the higher animals, including

man himself. They are known to bacteriologists as **pathogenic bacteria**, and they cause such virulent diseases as anthrax, erysipelas, tetanus (lock-jaw), typhoid fever, cholera, and tuberculosis. The bacteria that we shall study to-day are all harmless saprophytes engaged in the process of putrefaction (breaking down proteins, etc., into much simpler chemical substances).

The **Green Plants**, owing to their being able to carry on the process of photosynthesis, **build up vast masses of organic material** (sugar, starch, cellulose, wood, coal), while **Bacteria**, aided by **Fungi**, **destroy this organic matter** and finally reduce it to inorganic compounds which can be used as food by the Green Plants. Bacteria, therefore, are of great importance in maintaining the balance between the constructive and destructive forces in the organic world.

Bacteria, as a rule, are **very simple in outward form**. The chief forms are the **spherical**, the **rod-like**, and the **spiral**. Spherical forms are known as **cocci** (*Micrococcus*, *Streptococcus*), the rod-like forms may be very short to relatively long (*Bacterium*, *Bacillus*), and the spiral forms may be like a quarter of a turn of a cork-screw (*Vibrio*) or may be like a complete cork-screw with several or many turns (*Spirillum*, *Spirochaeta*).

Bacteria **increase in numbers** by a process of **simple fission**. A bacterium plant becomes constricted across the middle and the constriction increases until two daughter plants are produced. Each daughter plant then grows to the size of its parent and then divides in its turn. Under favourable conditions of nutrition and temperature one bacterium cell may divide into two cells **every half hour**. Owing to this rapid geometrical rate of increase in numbers, a single bacterium, within 24 hours, may give rise to many millions of descendants.

Bacterial Cultures for Class Use.—To obtain bacteria for examination, it is only necessary to expose water containing organic substances in solution (sugars, proteins, etc.) to the dust of the air, or to contaminate the solution with dirt, mud, dung, or some other substance in which bacteria are already present in large numbers. Every bacterium plant, on entering a fresh nutrient solution, absorbs food materials through its cell-wall, grows in size, and very soon divides by simple fission into two daughter plants; and each daughter plant grows and divides in its turn, and so on; so that, in the course of 2–3 days, a culture medium, e.g. a hay infusion, bouillon, milk, etc., which at first

had only a few bacteria in it, may come to contain tens of millions. In each of the cultures supplied to you to-day, the multiplication of the bacteria has been very active for the last two days and is still going on. Every drop of any of the culture media now contains tens of thousands or even millions of bacteria.

The Hay Bacillus in a Hay Infusion.—In hay there are large numbers of dried-up bacteria of different species adhering to the surface of the grass leaves and stems. Some of these bacteria are in the **vegetative condition**, while others are in the form of **spores** (resting reproductive bodies). Among these bacteria, chiefly in the form of spores, is *Bacillus subtilis*, the **Hay Bacillus**. This species is everywhere present in the uppermost layer of the soil in arable regions in both Europe and North America, so that it may be said to be one of the commonest of all bacteria. **The spores of *Bacillus subtilis* are highly resistant to heat**, whereas those of most other bacteria are much less resistant. With this fact borne in mind, a **hay infusion** (a decoction of hay) containing *Bacillus subtilis* was prepared as follows :

Some old hay was cut up and set in a beaker, and the beaker was filled with tap-water. Then the whole was boiled for ten minutes. As a result, organic substances came out of the hay into the water, thus turning the water into a brown **hay infusion** ; while, at the same time, most or all of the different kinds of bacteria on the hay and in the infusion were killed with the exception of the highly resistant **spores of *Bacillus subtilis***. The beaker, after it had cooled, was placed under a bell-jar, where it remained for about two days. The spores germinated and each one produced a bacillus, and the bacilli underwent repeated fission, so that now there are millions of Hay Bacilli in the culture to be examined.

Examination of a Hay-Bacillus Culture.—Place a slide on your desk number and a drop of the culture will be given you by one of the demonstrators.

Examine the preparation first with the low-power objective and then with the high. The **watery layer in which the bacteria are is very thin and transparent** and there may be some difficulty in finding it with the high-power. If so, proceed as follows. Bring one edge of the cover-glass into about the middle of the field of view and focus on it with the low-power. Then turn on the high-power and **find the edge of the cover-glass**. It is then easy by focussing to distinguish between the upper surface of

the cover-glass (by dust-particles on it, etc.) and the lower surface in contact with the liquid film. Another way to find the liquid film is by **focussing on an air-bubble** (first with the low-power and then with the high) and by moving the slide laterally.

Once more you are reminded of **the great importance of adjusting your light properly**. As usual, you should adjust your mirror with every care so as to get the **maximum amount of light up the tube** and an **evenly lighted field**. Then you should **cut down the light**, as required, **with the iris diaphragm**. With too strong a light, the bacteria will be invisible, so be sure and use the iris diaphragm in controlling the light, especially with the high-power.

When examining bacteria or anything else under the high-power objective, your **right arm should rest on the table** and your **first finger and thumb be kept on the micrometer head** of the fine adjustment. By focussing up and down slightly, you can examine different planes in the preparation and thus see more.

Find some of the Hay Bacilli. They are short rods, 0.7–1.2 microns in diameter and 2.5–9 microns long, and colourless. Owing to their very small size and want of colour, at first they may be overlooked; but, with careful focussing and adjustment of the light, they can be readily found.

Notice that there are a great many bacilli in the field of view at one time and that, while some of them are at rest, many of them are **motile**. In some of them the power of **locomotion** is very marked. Notice that an actively motile bacillus **moves in the direction of its long axis**—one end first. If it appears to oscillate from side to side as it progresses, that is because its course is in reality a **spiral** one. The motion is caused by the lashing of numerous **flagella (cilia)** which pass outwards through the cell-wall like little whips on all sides of the cell; but these flagella cannot be seen under present conditions. To make them visible it would be necessary first to kill the bacillus and then to stain the flagella by a special method in which a mordant is involved. If one were to measure the diameter of the field of view with the high-power and then to find out how long a bacillus takes to cross the field of view, one might calculate the speed of the bacillus. Relatively to its length, a bacillus moves far faster than a galloping horse.

If there is an **air-bubble** in your preparation (easily found with the naked eye, then with the low-power, then with the high), notice that the bacteria in the fluid **around its edge are much**

more motile than elsewhere. This is due to the fact that the movement of the bacteria is dependent on the presence of **oxygen** which these organisms, like other plants, use up in the physiological process of **respiration**. When bacteria are present in very large numbers (dense masses) in the drop under a cover-glass, they use up all the dissolved oxygen there present within a few minutes; and, as they exhaust the oxygen, they **come to rest**. But, around an air-bubble, the bacteria continue to move for a much longer time than elsewhere because they have **access to the oxygen diffusing out of the air-bubble**. Some bacteria **move toward an air-bubble** and gather around it in large numbers. This is called **aerotaxis** (oxygenotaxis), a particular case of the phenomenon known as **chemotaxis** in which a motile organism reacts to a chemical stimulus by changing its direction of locomotion. Most species of bacteria, but not all, are **positively aerotactic**.

Here and there you should find bacilli undergoing **simple fission** and showing a **constriction** in the middle of their cells. Sometimes, after a division has taken place, the two daughter-cells remain attached by their ends, and this may be repeated, so that a short **chain of Hay Bacilli** may be formed. The individual cells in such a chain readily separate from one another.

It is possible that some of the Hay Bacilli in your drop of culture medium may have formed **spores**. Each bacillus forms one spore only in its interior toward one end. A **spore** is distinguished by its **oval** or almost rounded form, its **thick cell-wall** and its **glistening appearance**. Spores retain their vitality, when dried, for months or years, and they are highly resistant to extremes of heat and cold. When placed in a nutrient medium, such as a hay infusion, they germinate within a few hours.

Make a drawing showing several Hay Bacilli, some **solitary** and some **in chains**. If you have seen them, add a bacillus undergoing **fission** and one or more containing **spores**. A single bacillus plant should be drawn about three-quarters of an inch long.

Examination of a Pea-seed Culture Medium.—Some dried peas were ground up and covered with water. There were bacteria adhering to each testa. The culture was left for 36–48 hours. It is now swarming with bacteria.

Examine a drop of the culture medium. Determine for yourself whether or not bacteria are present in it. Are there several species present mixed together? Can you observe **coccus forms** (spherical forms) as well as **rod-like forms**?

Examination of a Snail Culture.—Some pond snails were killed by putting them in boiling water. The water was then contaminated with bacteria contained in mud taken from an aquarium.

Examine a drop of the culture medium. **Try to find some spirilla.** A **spirillum**, e.g. of *Spirillum undula* or *S. tenue*, is known by its cork-screw-like form.

B. THE INTERNAL STRUCTURE OF THE STEM OF A DICOTYLEDON

General Remarks on the Stem of a Dicotyledon.—In the young stem of a Dicotyledon the vascular bundles are **few** in number, are **arranged in a ring**, and are provided with a **cambium**, so that the stem as a whole, after its first formation, can grow in thickness. There is **no** bundle sheath surrounding each bundle. The dicotyledonous stem is also characterised by having a central **pith** (medulla), **medullary rays** and, if it is perennial (shrubs and trees), **annual rings of wood**. By counting the number of yearly rings in a cross-section of a dicotyledonous tree trunk, one can estimate the age of the tree. All the trees and shrubs in Manitoba, e.g. Elm, Manitoba Maple, Blueberry bush, are Dicotyledons. Monocotyledonous trees, e.g. Palms, Dragon trees, Yuccas, flourish only in tropical and subtropical countries. A vascular bundle of a Monocotyledon, since it has no cambium and therefore no possibility of further growth, is said to be **closed**; whereas a vascular bundle of a Dicotyledon, since it has a cambium which may add new cells to the phloem and xylem, is said to be **open**. Since in both Monocotyledons and Dicotyledons in every vascular bundle the phloem and xylem are side by side on the same radius, the bundles in these groups are said to be **collateral**.

The structure of a dicotyledonous stem will now be studied with the help of transverse sections taken from (1) a Castor Oil plant and from (2) a Basswood tree.

C. TRANSVERSE SECTION OF A YOUNG STEM OF RICINUS COMMUNIS IN WHICH A CAMBIUM RING HAS JUST DEVELOPED

Section A.—*Ricinus communis* is the **Castor Oil plant**. The origin of Section A was as follows: A Castor Oil seed was planted in a pot. It germinated and produced a seedling. The seedling raised its two cotyledons into the air and they became the first two green leaves. The piece of stem below the up-raised cotyledons connecting them with the root is known as the **hypocotyl**. In this young stem there were at first eight vascular bundles

but no cambium. Then the cambium developed in each bundle between the phloem and the xylem (**fascicular cambium**) and across each medullary ray (**interfascicular cambium**), so as to form a **cambium ring**. At this stage of development, the stem was preserved by placing it in alcohol; and, subsequently, the section provided was cut from it by hand, stained, and mounted permanently in Canada balsam.

Section B.—A second permanently-mounted preparation of a young *Ricinus* stem with a cambium ring is provided. This was cut with the help of a microtome and is therefore both even and complete. It shows the bast fibres better developed than they are in Section A, but in it in general the cell-walls of the parenchymatous cells, etc., are unnaturally wrinkled, and the cell-contents are absent (due to mode of making the preparation).

Macroscopic Examination of the Preparations provided.—With the naked eye, examine the preparations against a white background (white paper, light from a window) and observe the broad **cortex** stretching from the epidermis to the **central cylinder**. Notice that the **central cylinder** consists of **eight** well-separated wedge-shaped **vascular bundles** embedded in **ground tissue**, and that the ground tissue consists of a large central **pith** or medulla and of **eight primary medullary rays**, each ray radiating outwards from the pith between two adjacent vascular bundles and connecting the pith with the cortex.

Microscopic Examination of the Preparations.—With the low-power objective explore the sections and make out as many of the details of structure as possible. The following is a list of all the tissues and cell-structures which are present in such cross-sections as those provided.

An Analysis of the Tissues of a Young Castor Oil Stem.

- A. **Epidermis**—one layer of cells thick, cells rather small.
- B. **Cortex**.... many layers of cells thick, cells larger parenchymatous.

C. Central Cylinder (Stele) :

- | | | |
|----------------------|---|--|
| I. Ground Tissue.... | } | Pith—in the centre, made up of large parenchymatous cells.
Medullary rays—8, connecting pith and cortex, made up of large parenchymatous cells. |
|----------------------|---|--|

II. Vascular Bundles. .8, wedge-shaped, in a ring :

- | | | |
|---------------------|---|---|
| 1. Phloem (bast)... | { | Bast fibres—the outer cells next to cortex, becoming thick-walled. |
| | { | Sieve-tubes } few in num-
Companion } ber, in small
cells } groups. |
| | { | Bast parenchyma — the majority of the phloem cells. |
| 2. Cambium..... | { | Seen as a ring of meristematic tissue running between the phloem and xylem of each bundle and across each medullary ray, known by its tangential cell-walls which have recently been produced by cell-division. |
| | { | Spiral vessels—few in number and small, the first-formed xylem elements, nearest to centre of the stem, at the base of the rows of pitted ducts. |
| | { | Scalariform vessels — with ladder - like thickenings, next to the spiral vessels. |
| 3. Xylem (wood).. | { | Pitted ducts—known by their large size, they are arranged in a few radial rows. |
| | { | Thick-walled smaller cells—some of these may be tracheides, others parenchymatous cells, and others possibly wood fibres. |

D. **Latex Vessels**—isolated cells, made conspicuous by their dense well-stained cell-contents, scattered about in the epidermis, cortex, phloem, and pith. They are branches of latex vessels which together make up a special system known as a **latex system**, present only in relatively few

plants. During the growth of the stem the latex-tube branches pushed their way up between the other cells into the positions which they now occupy.

In studying the stem with the microscope, make out : (1) the **epidermis**, the **cortex**, and the **central cylinder** as a whole ; then (2) the eight **vascular bundles** as wholes, the **pith**, and the eight **medullary rays** ; and, finally, (3) the **phloem**, the **cambium ring**, and the **xylem**. The **latex vessels**, referred to in the "Analysis of the Tissues," should also be noticed. There is more or less difficulty in making out the various kinds of elements which enter into the structure of the phloem and xylem.

The **cambium ring**, running between the phloem and xylem of each bundle (**fascicular cambium**) and across each medullary ray (**interfascicular cambium**) should be traced all around the section with the low-power objective, and then favourable portions of it should be studied with the high-power. Notice that the cambium ring is only a **few cells thick**, that its new walls are **tangential** in direction (parallel to the epidermis), and very **thin**. The **initial cells** of the cambium **stretch in the radial direction** and then become divided by tangential cell-walls. The cambium ring in your preparation, since it has only just been formed, has not yet produced any secondary ring of xylem on its inside nor any secondary phloem on its outside, but it is now ready to produce these secondary tissues.

The **phloem** (bast) is the outermost part of each vascular bundle and bulges out beyond the cambium into the cortex. The outermost cells of the phloem become thick-walled **bast fibres** set closely together without any intercellular spaces between them. Their walls in your sections are in process of receiving their secondary thickenings. In Section B the bast fibres are further advanced than in Section A and can be readily recognised. Between the bast fibres and the cambium are numerous **bast parenchymatous cells** among which, here and there, are a few small **groups of sieve-tubes and their companion cells**. It is somewhat difficult to distinguish all these thin-walled cells from one another ; but the companion cells are known by their small size, and each companion cell has a larger sieve-tube at its side. When a vascular bundle is developing, a single small companion cell is always cut off from a cell which is to become a sieve-tube. While a sieve-tube is believed to conduct organic substances

manufactured in the leaves, the function of the companion cells is unknown, but it is supposed that the companion cells assist the sieve-tubes in some way. All the phloem in your sections is **primary phloem**. **Secondary phloem** is formed by the cambium, but as yet there is none in your section.

The **xylem** (wood) is the innermost part of each vascular bundle and stretches from the cambium into the pith. It is **primary xylem**. **Secondary xylem** is formed by the cambium as a ring or rings of wood, but there is none in your section. In the xylem, from the pith outwards, there are a few rows of larger elements. The innermost of the cells in the rows are small **spiral vessels** (with spiral thickenings), then come one or two larger **scalariform vessels** (with ladder-like thickenings), and then two or three large **pitted ducts** (with small pits on the walls). Between and at the sides of the two or three radial rows of wood vessels are a number of **thick-walled smaller cells**, the nature of which it is almost impossible to determine in a transverse section. Some of them may be **tracheides** (long pointed elements with bordered pits), some **wood parenchymatous cells** (short living cells), and some possibly **wood fibres** (long pointed elements with simple pits). While the wood vessels serve to conduct water with substances in solution up the stem, the smaller thick-walled cells increase the mechanical strength of the wood.

Make a semi-diagrammatic drawing showing the position of the **epidermis**, the **cortex**, the **central cylinder**, the **cambium ring**, the **phloem** and the **xylem** of each of the eight **vascular bundles**, the **pith**, and the eight **medullary rays**. Make a circle with the help of the cover-glass dish and mark its central point. Let the circle represent the epidermis. Then look at one of your preparations with the naked eye and from it draw in the eight vascular bundles and the cambium ring. On the small scale employed the individual cells cannot all be put in in detail; but, if you so desire, you may indicate, say, a few epidermal cells (about six), a narrow radial strip of cortical cells, the radial rows of pitted ducts in one or more of the vascular bundles, some medullary ray cells, and a narrow radial strip of pith cells. It would take too long to do more than this in this practical period. As usual, indicate what your drawing represents and the names of its various parts.

- V. Cambium in form of a cambium ring, 2-3 cell-layers thick, between the phloem and xylem.
- VI. Xylem (wood) . . secondary (the 4-5 annual rings of wood, the youngest next to the cambium, each ring with larger-celled spring wood and smaller-celled autumn wood); and primary (shallow masses of small cells, at intervals projecting slightly into the pith).
- VII. Medullary rays . . strands of cells running radially through the xylem and phloem. They are parts of these tissues. A few open out funnel-wise in the phloem.
- VIII. Pith (medulla) . . the parenchymatous cells in the middle of the stem.

Notes on the Tissues summarised above.

I. **The Epidermis.**—This is broken away in many places owing to the growth in width of the stem. Its fragments are difficult to find. A layer of cork has now taken on its protective function, thus reducing its importance.

II. **The Cork.**—A thin layer of dead brown cells immediately under the epidermis. It is several cells thick and each cell is much compressed in the radial direction. The cells are arranged in radial rows, and each row looks like a pile of books seen from one side. Except at places known as **lenticels** (probably there is not one in your section) there are no intercellular spaces between the cork cells. Cork is impermeable to air and water and is very tough. It is a **secondary tissue**, never present in a very young stem, and it is produced by a special cambium of its own known as the **cork cambium**; but, for observing a cork cambium, a Basswood stem is not favourable material.

III. **The Cortex.**—This lies under the layer of cork and consists of thin-walled parenchymatous cells several layers thick. Within, it is in contact with the outermost fibres of the phloem and with the funnel-like outer parts of certain medullary rays. The cortex is somewhat distorted owing to strains set up by the thickening of the stem as a whole. Many of its cells are specialised as

crystal-sacs, each of which contains a **cluster-crystal** of **calcium oxalate**. Each crystal is rather star-like in form, and must be considered as a useless waste product. It appears that oxalic acid is one of the products of metabolism and that it is rendered harmless by being combined with calcium to form a salt which is insoluble in water. Raphides and cluster-crystals are both made of calcium oxalate, but they have crystallised out in two different ways. Some of the large cortical cells are **mucilage-sacs**. They contain **mucilage** which in some sections may be deeply stained.

IV. The Phloem (Bast).—This stretches from the cortex to the cambium which alone separates it from the xylem. It is a fairly thick layer. It is made up of **several layers of bast fibres and of thin-walled cells**, the layers alternating with one another; and **funnel-like expansions of certain of the medullary rays** can be seen running through it from the cambium to the cortex. The **bast fibres** in a bast-fibre layer are distinguished by their small size, their thick strongly-stained cell-walls (the lumen in each cell can hardly be seen, owing to the thickening of the cell-wall it has become reduced to a mere point), and by fitting together very compactly. They strengthen and protect the phloem mechanically. Similar bast fibres occur in the phloem of flax and hemp stems, from which they are extracted to make linen goods and hempen ropes, etc.

The layers of thin-walled cells alternating in the radial direction with the layers of bast fibres consist of: (1) **sieve-tubes**, larger, clearer cells in groups of about 3–4, each sieve-tube with a tiny **companion cell** cut off at one of its ends; and of (2) **bast parenchymatous cells**. These are all the thin-walled cells around the groups of sieve-tubes and companion cells.

The **primary phloem** which was present in the stem before the cambium was formed lies next to the cortex, while the **secondary phloem** (most of the phloem) which was formed by the cambium lies next to the cambium. Primary and secondary phloem are **exactly alike** in appearance, and where one passes into the other cannot be determined.

Although the cambium forms a certain small amount of new phloem on its outside each year, it never gives rise to definite annual rings of phloem easily distinguishable from one another with the naked eye or with the microscope. In having no annual rings secondary phloem differs greatly from secondary xylem.

V. **The Cambium.**—This can be seen as a complete ring of delicate thin-walled cells, about 2–3 layers of cells thick, between the phloem and the xylem. It has effected all the secondary thickening of the phloem and xylem. It is so delicate that, in cutting hand-sections, the outer tissues often split away from the wood along the line of the cambium. It is active in spring, summer, and early autumn, and it rests through the winter. It is more active on its inner side than on its outer. Hence the **amount of secondary xylem which it produces always greatly exceeds the amount of secondary phloem.** In the spring it produces wood cells which are **wide** in the radial direction, and in the autumn wood cells which are **compressed** in the radial direction. Hence the contrast between the autumn wood and the spring wood of the next year which allows us to perceive the limits of each annual ring of wood. When a cambium **initial cell** is active, it first **stretches in the radial direction** and then undergoes cell-division by means of a **tangential wall**. Then one of the daughter-cells becomes an initial cell, and so on. The innermost cambial cells thus produced become converted into wood cells, while the outermost ones become converted into phloem cells. In some trees the cambium, after its formation as a ring in the main stem, remains active for hundreds of years.

VI. **The Xylem (Wood).**—This stretches from the cambium to the pith and consists of **about five annual rings of secondary xylem** and of some little **shallow masses of primary xylem** projecting into the pith. **Primary xylem** is the original xylem of the vascular bundles of the very young stem. **Secondary xylem** is the xylem added to the exterior of the primary xylem by the action of the cambium. The little masses of primary xylem projecting slightly into the pith are composed of small cells and their individuality cannot now be well made out. In the secondary xylem the innermost ring of wood was formed in the first year and its first cells were formed by the cambium ring as soon as this came into existence. The outermost yearly ring is the one which was last formed by the cambium.

In each annual ring of wood the innermost cells were formed in the spring of the year as soon as the cambium resumed its activity, the middle part of the ring was formed during the summer, and the outermost part of the ring during the autumn. The spring wood in the Basswood always contains many wood vessels (pitted ducts); there are fewer vessels produced as the

summer progresses, and none produced in the autumn. The **spring wood** is composed of cells well **stretched out in the radial direction**, while the **autumn wood** is composed of a few layers of cells strongly **compressed in the radial direction**. Notice the contrast between the autumn wood of one year and the spring wood of the next year. It is this contrast which enables us to count the yearly rings of wood in a cross-section of a tree trunk.

The elements of the wood in the annual rings are often arranged more or less in radial rows, each row corresponding to one cambial cell.

The kinds of cells in the annual rings of wood are, exclusive of medullary-ray cells, as follows : (1) **Wood vessels** (pitted ducts, etc.) known by their large size ; (2) **Tracheides**, long pointed elements with bordered pits, difficult to tell in cross-section, but to be recognised by their small size as compared with the wood vessels, by their pits, and by their lack of cell-contents ; (3) **Wood Parenchymatous Cells**, small cells, when alive with protoplasmic contents and starch, etc. ; and (4) **Wood Fibres**, elongated fairly thick-walled cells, without contents and without bordered pits. The wood vessels and tracheides **conduct** water, the wood parenchymatous cells **store** up starch, etc., during the winter, while the wood fibres together with all the other elements give **mechanical strength** to the wood.

VII. Medullary Rays.—These consist of rows of cells running radially through the xylem and phloem of which they form a part. The cells in a medullary ray are **elongated not in the longitudinal direction of the stem but in a radial direction**. The cells are living. Their functions are not fully understood, but it is certain that the medullary rays usually **store up starch or oil** during the winter. They probably play an important part in bringing food substances to the cambium and they may have something to do with the ascent of the sap in the wood.

The **primary medullary rays** are those which run from the pith to the cortex and open out in a funnel-wise manner as they pass through the phloem. The other medullary rays originated later by the action of certain cambial cells and they are therefore **secondary medullary rays**. These secondary medullary rays are mostly only one layer of cells wide both in the wood and in the phloem. Some of them are much shorter than others. The shorter a medullary ray, the later did it begin its existence.

VIII. The Pith.—This occupies the centre of the stem and

consists of parenchymatous cells resembling those of the cortex. Some of the pith cells are specialised as **mucilage-sacs**. These are fairly large cells containing **mucilage** which, if still present, will be deeply stained. It will be remembered that mucilage sacs are also formed in the cortex.

Drawing.

Make a drawing of a sector of the stem (an arc of the epidermis joined by straight lines to the centre of the pith). The width should be 3-4 inches and the length about 6 inches. After drawing the outline of the sector, put in the **cambium** in its right relative position, then the **pith**, then the **annual rings of wood**, then the **phloem** and **large medullary rays**, then the **cortex**, **cork**, and **epidermis** (if any of it has been seen by you). Show the cell-structure of the various tissues as well as you can. Add names to each part.

CHAPTER XVIII

A WHITE MOULD. THE STRUCTURE OF WOOD

Materials and preparations supplied :

- (1) A Mould fungus, *Rhizopus nigricans* or *Mucor mucedo*, 3-4 days old, growing on damp bread in a crystallising dish. Dish now upside down to preserve the moisture.
- (2) Germinating spores of a Mould fungus in a drop of nutrient gelatine under a cover-glass in a ring-cell. The nutrient gelatine consists of: 100 c.c. water, 1 gram peptone, 0.5 gm. sodium chloride, 0.5 Liebig's beef extract, and 10 grams gelatine. Spores sown yesterday evening.
- (3) A ring-cell, with a drop of water at its base and a hanging drop of nutrient gelatine on the under side of the cover-glass. Spores sown two days ago. They germinated and have now produced mature fungus plants showing both mycelium and sporangia.
- (4) A rectangular block of Oak wood, for the study of annual rings and medullary rays in cross and longitudinal sections.

- (5) An Oak-wood stick, for an experiment with air and water.
- (6) A transverse section of the trunk of a Tamarack or Pine tree, for a study of the annual rings and position of the cambium and phloem.
- (7) A rectangular block of Douglas Fir wood, for the study of annual rings in cross and longitudinal sections.
- (8) A stick of Jack Pine wood, for an experiment with water and air.
- (9) A microscope slide showing transverse and longitudinal sections of the wood of the Douglas Fir.
- (10) A microscope slide showing transverse and longitudinal sections of the wood of the Scots Pine.

A. MOULD FUNGI

General Remarks.—Fungi form a large group of Flowerless Plants (Cryptogams), and they include Mushrooms and Toadstools, Bracket Fungi on tree trunks, Puff-balls, Rust Fungi, Smut Fungi, Mildews, Moulds, and Yeasts. They differ from the Algae (Protococcus, Closterium, Spirogyra, etc.) in not possessing any chloroplasts or chlorophyll. Owing to this lack of chlorophyll, fungi are **unable to carry on the process of photosynthesis**, i.e. they cannot manufacture sugar from carbon dioxide and water with the help of energy derived from sunlight. This being so, fungi are dependent for their existence on complex organic matter (proteins, sugar, cellulose, etc.) built up originally by the green plants or by animals that have fed directly or indirectly on green plants. A fungus which lives on dead organic matter (e.g. the Common Mushroom) is known as a **saprophyte**; while a fungus which lives on a living plant (e.g. a Rust fungus on a Wheat plant) or on a living animal (e.g. the Ringworm fungus on the scalp of a human being) is known as a **parasite**. The Mould fungi are saprophytes.

Moulds are commonly found on organic substances kept sufficiently moist, e.g. old bread, old tea-leaves, fruits of various kinds, dead leaves, dead sticks, horse dung, etc. They excrete enzymes (diastase, proteolytic enzymes, etc.) from their cells which act on these substances and change their insoluble parts (starch, proteins, etc.) into soluble compounds which may be absorbed in solution, and they cause these organic substances on which they feed to go mouldy. In the process various volatile chemical compounds are set free, and it is these emanations

which give to mouldy bread, etc., its characteristic unpleasant mouldy odour. Among the commonest Mould fungi are : *Mucor mucedo*, the White Mould or Pin Mould, which comes up on horse dung ; *Rhizopus nigricans*, the Bread Mould, which often appears on damp bread ; and *Penicillium glaucum* and *Aspergillus herbariorum*, both of which are Blue-green Moulds. *Penicillium glaucum* attacks a great many different organic substances, to which its green spores give a peculiar glaucous-green colour (e.g. bread, tea-leaves, leather boots).

Rhizopus nigricans, the **Bread Mould**.—This fungus flourishes on starchy media and, in particular, on damp bread. Hence its common name. It can be obtained in the laboratory by exposing damp bread to the air and by then putting the bread in a closed glass chamber. Spores of the fungus fall on to the bread from the air, germinate on the bread, and the vegetative body or **mycelium** of the fungus thus produced grows over the bread and ramifies in its substance, thus causing the bread to go mouldy. Then certain branches of the mycelium are sent up into the air. These aerial branches (hyphae) terminate in tiny spherical black **sporangia** and in each sporangium there are some hundreds of **spores**. The spores serve to disseminate the fungus and to provide for its reproduction.

Examination of *Rhizopus nigricans* growing on Bread.—Examine the bread culture provided. The small crystallising dish which contains it is kept upside down to prevent the mould suffering from drought. It may be turned the right way up for temporary examination. Some spores of the Bread Mould were sown on the bread 3–4 days ago.

The Bread Mould, like all other fungi except the Yeast plant, consists of two distinct parts : (1) the **vegetative** and (2) the **reproductive**. Examine the Bread Mould with the naked eye and try to make out the two parts of the plant, which will now be described in detail.

(1) The **vegetative part** of the Bread Mould is **unicellular**. It constitutes the plant-body and is known as the **mycelium**. The mycelium is made up of delicate white filaments or **hyphae** (sing. *hypha*) which grow into and over the bread and over the damp surface of the glass. The hyphae in this particular mould are of three kinds : (a) **ordinary hyphae** which form parts of the mycelium developed from a spore, (b) hyphae known as **stolons**, and (c) other hyphae known as **rhizoids**. The **ordinary hyphae**

are much branched, thin, colourless, cylindrical organs which are derived from the branching of a germ-tube of a spore ; and they grow into the bread and attack it. A **stolon** is a thick and rather long, cylindrical, unbranched arched hypha, which grows out from the mycelium into the air for some distance over the substratum (*cf.* the runner of a strawberry plant), touches the substratum again with its apex, and then, as it were, takes root again, thus providing for the rapid spread of the mycelium in a lateral direction. Wherever a stolon touches the firm substratum (bread, glass) it sends down to the substratum a delicate rosette of fine root-like hyphae which are the **rhizoids** already mentioned. Collectively the rhizoids may be regarded as an **appressorium** or organ of attachment. From above a group of rhizoids one or two new stolons are sent out, and thus the mycelium creeps forward rapidly, spreading itself radially very much like a Strawberry plant does by means of its runners. The rhizoids, if produced on a nutrient substratum such as damp bread, may grow down indefinitely into the substratum and feed upon it.

(2) **The reproductive part** of the Bread Mould consists of numerous **sporangia** borne on **sporangiophores**. A group of about 2-6 of these structures is produced above each appressorium (group of rhizoids) and they project outwards into the air, diverging from one another, like pins on a pin-cushion. Each sporangium is **at first white**, but it becomes **black** as it matures and it can then be seen with the naked eye as a **small black spherical structure** supported on a long sporangiophore. A sporangium is always cut off from its sporangiophore by a curved wall in such a way that the sporangiophore projects into the sporangium to form what is known as the **columella**. The sporangium is bounded externally by a thin, firm, but very delicate **sporangium wall** which encloses numerous tiny **spores**. The spores are packed closely together between the sporangium wall and the hemispherical columella. Each **spore** is rounded in form and covered with a thick grey-brown cell-wall ; and it is the dark spores which collectively give the ripe sporangium its black colour.

A **spore differs from a seed** in being unicellular and in not containing any embryo. A spore, therefore, is a much simpler reproductive body than any seed.

The vegetative and the reproductive parts of the Bread Mould **change colour with age**. At first they are pure white but, as

they grow older, they become yellowish-brown or dirty brown, and this applies even to the columella.

The Bread Mould, until each sporangium is cut off from its sporangiophore by a cell-wall, is a **unicellular plant**, all its hyphae being only branches of a single cell. Evidently, in its structure, the Bread Mould is far in advance of the other unicellular plants already studied, namely, *Protococcus*, *Closterium*, Yeast, and Bacteria. It presents a good example of what is known as the **morphological differentiation of structure** and the **physiological division of labour**. Whereas in *Protococcus* and Yeast the entire organism performs all the functions of life, in the Bread Mould one part of the plant performs one function (the mycelium penetrates into the substratum and feeds upon it) and another part of the plant performs another function (the sporangiophores and sporangia grow at the expense of food materials sent to them by the mycelium and provide for reproduction); and at the same time each part of the plant has undergone modification in form and structure, thus fitting it for its particular work to be done in the service of the organism as a whole.

Examination of a Hanging Drop containing Germinating Spores.—A glass ring was attached by means of paraffin wax to a glass slide, a drop of water was placed in the bottom of the ring, then some mould spores were sown in a tiny drop of nutrient gelatine set on a cover-glass. Then some vaseline was placed all over the upper edge of the glass ring. Finally, the cover-glass was turned upside down and set on the ring, so that now you are provided with a hanging drop of nutrient gelatine containing mould spores. The drop cannot dry up as it is in a little damp-chamber, and the spores have access to oxygen since air is enclosed in the chamber. The piece of apparatus, as it now stands, is often called a **van-Tieghem cell**, after van Tieghem, a botanist who first made use of it.

The spores were sown in the hanging drop late yesterday, and now, in all probability, many of them have germinated.

Examine the preparation first with the low-power objective and then with the high. **Be careful not to smash the cover-glass** by pressing the high-power objective down upon it.

Some of the spores have not yet begun to germinate, but others exhibit the first stages in the process. A spore, on germinating, swells up greatly and then puts out one or two **germ-tubes**. Look for swollen spores and their germ-tubes.

Make a sketch showing one or two germinating spores. Indicate the germ-tube or germ-tubes as seen.

Examination of a Full-grown White Mould Plant which has developed in another Hanging-drop Culture.—Another hanging-drop culture made like the first is provided; but the spores were sown in the nutrient gelatine **two days ago** instead of yesterday afternoon. Hence **the Mould** has had a longer time to develop and **is now full-grown**. Many mould fungi develop so rapidly that they can pass through all the stages of their life-history from spore to spore in 24–48 hours. The nutrient substances in the hanging drop are peptone, common salt, and Liebig's beef extract. The gelatine is added not as a food substance, but as a solidifying substance. The Mould species from which the spores were obtained was either *Mucor racemosus* or *Mucor mucedo*.

With the **low-power** objective examine the preparation and observe (1) the **mycelium** growing just under the cover-glass in the hanging drop and over the damp surface of the glass, and (2) the **sporangia** borne on **sporangiphores** which project downwards from the mycelium into the air of the chamber. To see the sporangia, it is necessary to focus downwards through the air of the chamber.

With the **high-power** objective it is possible to examine the mycelium more in detail; but it is impossible to focus downwards to the sporangia without breaking the cover-glass, so do not attempt this. **Be careful not to smash the cover-glass.**

Make a drawing showing semi-diagrammatically a branched mycelium bearing one or more sporangiphores and sporangia.

Examination of a Sporangiphore and Sporangium of the Bread Mould.—With a needle or knife remove a group of sporangia from the large bread culture in the crystallising dish and place it on a slide. Do **not** add any water but lay on a cover-glass. Examine with the microscope. You will probably see some of the sporangia still unbroken.

Now **run some water under the cover-glass** and observe its effect under the microscope. Probably you will find that the **wall of each sporangium**, on coming into contact with the water, swells up and **disappears**, thus exposing the dark **spores** to view. The hemispherical **columella** at the top of the sporangiphore does not dissolve but is left behind, and you may see numerous spores still clinging to its outer surface.

Make a semi-diagrammatic drawing of the Bread Mould showing

one or more **stolons**, some **rhizoids** (appressorium), and one or more groups of intact **sporangia** on their sporangiophores. A stolon may be made 2-3 inches long and then everything else in proportion to it.

Make a second drawing showing a burst sporangium. Put in the upper part of the **sporangiophore** ending in the **columella** and a number of **spores**.

B. OAK WOOD

Examination of a Rectangular Block of Oak Wood.—With the naked eye, in the block of Oak wood supplied, make out in **transverse section** the **annual rings of wood**. Notice that in each ring there are numerous **wood vessels** (pitted ducts), and that the vessels are of two sizes : (a) very large vessels, 3-4 deep, forming a definite readily recognisable layer in the **spring wood**, and (b) much smaller vessels scattered through the **summer** and **autumn wood**. The only part of an annual ring in which vessels are entirely absent is the last-formed autumn wood, a zone of the ring not more than about 0.5 mm. wide. **Between the vessels** are dense masses of wood made up of cells which are so small that they cannot be distinguished with the naked eye. These cells include groups of **tracheides**, strings of **parenchymatous cells**, and large numbers of thick-walled **wood fibres**. It is the wood fibres in particular which give to Oak wood its great strength.

In the transverse section notice further that running in what was the radial direction of the tree trunk, and therefore crossing the annual rings of wood at right angles, are a number of **broad light-brown medullary rays**. These broad rays are **several layers of cells** in width. Between the relatively few broad medullary rays are more numerous **very much narrower medullary rays**. The latter cannot be seen with the naked eye, as they are only about one layer of cells wide.

The block may be **placed upright on the stage of the microscope** and its upper transverse section be examined with the **low-power** objective by reflected light. Then the following structures can readily be observed : (1) the very large spring wood vessels, (2) the distinctly smaller summer and autumn wood vessels, (3) the broad medullary rays, (4) between any two broad medullary rays, several narrow medullary rays, and (5) dense woody tissue filling up the spaces between the vessels and

medullary rays, the cell-elements of which are so narrow that they cannot be distinguished one from another.

Examine the **longitudinal surfaces** of the block. Notice that the **vessels**, particularly the very large ones of the spring wood, can be seen as **hemi-cylindrical grooves**, and that with their help the annual rings of wood can be made out. Notice, further, the broad **medullary rays**. These should be traced from the transverse section down the sides of the block. It will then become clear that every single medullary ray is a vertical ribbon-like plate of tissue from about one-quarter up to three-quarters of an inch high, running radially through the annual rings of wood. In Oak wood the medullary rays are particularly well-developed in height and thickness, with the result that in **polished Oak furniture they are seen as prominent broad streaks or markings usually running obliquely across the annual rings of wood**. The exact appearance of the medullary rays in furniture must, of course, depend on exactly how the boards were cut out of the tree trunk whence they originated.

In cutting wood with an axe, as everyone knows, one can more or less readily split any kind of wood along the grain, i.e. in the longitudinal direction of the tree trunk, but one cannot split wood across the grain, one must always **cut** wood in the transverse direction. A knowledge of the minute structure of wood should enable you to explain the technique of wood-cutting, whether with an axe or a carpenter's plane, in terms of the form and distribution of wood cells.

Make a drawing of one-half of a transverse section of the block of Oak wood twice the natural size. Indicate the **annual rings**, the **spring wood** of a ring with its large wood vessels, the **summer** and **autumn wood** with their smaller vessels, and the broad **medullary rays**. The spring wood vessels should be put in with little circles, the other vessels with small dots, and the medullary rays by lines.

A Stick of Sap-wood of an Oak.—Notice the wood vessels as seen in cross-section. **To prove that the wood vessels** (each of which consists of a row of cells which have fused together) **are continuous pipes** which run right through the stick from one end to the other, put one end of the stick in your mouth and the other end in the water in your tumbler and blow air-bubbles through it. If you have clean water in your tumbler, you might try to suck some water up through the vessels into your mouth. This

can be done with great ease. These experiments throw some light on the problem of the ascent of sap in tall trees.

In many Oaks the older vessels, which are farther from the cambium ring, become stopped up by means of curious ingrowing cells called **tyloses** (sing. *tylosis*). Tyloses are formed by the living parenchymatous cells which surround each vessel. These living cells grow through the pits on the sides of the vessels and the ingrowths so formed stretch across the vessels and completely block them up. These ingrowths, when fully formed, are the tyloses. It is impossible to blow air through the heart-wood of any Oak in which tyloses are formed.

C. THE WOOD OF THE CONIFERAE

General Remarks.—The **Coniferae** or cone-bearing trees, e.g. **Pine, Spruce, Tamarack**, have relatively soft and even wood which is easily cut with a saw and worked with a plane. This is due to the fact that the secondary wood of the Coniferae, i.e. the annual rings of wood, consists (excepting resin-ducts) of two kinds of elements only, **tracheides** and **medullary-ray cells**, whereas in the secondary wood of Dicotyledons (hard-wood trees, e.g. Oak, Maple) in addition to tracheides and medullary ray cells there are present wood vessels, wood fibres (often with very thick walls), and wood parenchymatous cells.

Examination of a Transverse Section of a Trunk of a Tamarack. The Tamarack is also known as the **Larch**. In Canada there is only one species, *Larix laricina*, which is a slender tree found chiefly in cold swamps. Its wood is very resinous, owing to the presence of numerous resin-ducts in it. Unlike our other Conifers, the Tamarack is not an evergreen, but drops its leaves in the autumn and forms new ones in the spring. Its bark is distinctly reddish.

Examine the section provided. How old was the tree when it was cut down? Estimate its age by counting its annual rings of wood. Record your answer in your book.

Notice that in each ring of wood the **spring and summer wood** are whitish or light brown, whereas the **autumn wood** is deep brown or reddish in colour. The autumn wood is denser and harder than the spring wood, and this is indicated to some extent by its resistance to being torn by the plane. There are numerous but very thin **medullary rays** running radially across the annual

rings of wood, but they are so fine that with the naked eye you may not be able to detect them. Try to make them out. Notice the **absence** of any wood vessels and that the wood appears to be very even in texture.

Observe that the wood is divisible into sap-wood and heart-wood. The **heart-wood** is centrally situated and distinctly redder than the broad **sap-wood** which surrounds it. Heart-wood grows gradually in width at the expense of the sap-wood. All its cells are dead, and it does not conduct water up the stem.

The other tissues in the cross-section are : (1) a small central **pith**, no larger than the dot of the letter *i* ; (2) the **cambium**, too thin to be seen as such, it lies just outside the last ring of wood, and in places, owing to its delicacy, it has formed a line of cleavage, along which the outer tissues have split away from the wood ; (3) the **phloem**, outside the cambium, deep-brown in colour, extremely thin, not more than about 0.5 mm. wide in the radial direction ; and (4) the scaly **bark**, only a few mm. thick, deep brown, the same colour as the phloem, from which it can be told by its more external position and its scaliness. The bark consists of a series of thin layers of cork with old dead phloem in between. New bark in the Tamarack is constantly being formed at the expense of the older outer phloem cells. The epidermis and cortex have long disappeared. Each **scale of bark** is nothing more than a more or less flattened lenticular mass of dead phloem cells enclosed in front and behind by an extremely thin layer of cork. In the course of years, as the outer bark scales off, a considerable amount of the older phloem is gradually removed from a tree. The reddish colour of the bark is due to the colour of the cell-walls.

When one compares the radial width of all the secondary tissues **outside** the cambium (phloem and bark) with the radial width of all the secondary tissues **inside** the cambium (the annual rings of wood), it is evident that **the cambium** in the course of its many years of activity **forms new wood cells on its inside much more rapidly and abundantly than it forms new phloem cells on its outside.**

Examination of a Rectangular Block of Douglas Fir Wood.—The Douglas Fir, *Pseudotsuga mucronata*, occurs from the east slope of the Rocky mountains in Alberta through to the coast in British Columbia. It sometimes attains a height of 350 feet

and a diameter of 10 feet. There is a noble group of giant Douglas Firs in Stanley Park, Vancouver.

In transverse and longitudinal sections make out the **annual rings of wood** and, in each ring, distinguish between the **lighter brown softer spring and summer wood** and the deeper brown or **red, denser and harder autumn wood**. The contrast between the two kinds of wood in each annual ring is particularly well marked in the Douglas Fir. In future, when you look at varnished coniferous wood of the Douglas Fir type, remember that the **lighter and darker streaks which alternate** with one another are due to **lighter-coloured spring and summer wood alternating with darker-coloured autumn wood**. Notice the absence of wood vessels.

The block may be **placed upright on the stage of the microscope** and its upper transverse section be examined with the low-power objective by reflected light. Then it will just be possible in the **softer spring and summer wood** to detect some of the numerous very narrow **medullary rays** (they look like ridges) and also perhaps some of the individual **tracheides**. Plane-marks have been left on the dense autumn wood: do not mistake these for medullary rays. The medullary rays are only one layer of cells wide.

Make a drawing showing both the upper and a side view of part of the block. Indicate the **annual rings** and, in each ring, the lighter **spring and summer wood** and the darker **autumn wood**.

A Stick of Jack Pine Wood.—Once more notice the annual rings of wood with alternating lighter and darker parts in each ring. Repeat the experiment made with the Oak stick. Put one end of the Pine stick in your mouth and the other end in water and attempt to blow air-bubbles through it. Any failure to accomplish this is due to the fact that the longitudinally-stretched elements in the wood are all **tracheides** (closed pointed cells), no wood vessels being present in any of the annual rings.

Microscopic Examination of the Wood of a Conifer.—Two sets of sections are provided: (1) of Douglas Fir, and (2) of Scots Pine. Under each cover-glass there are three sections: (a) a **transverse** section, (b) a **longitudinal-radial** section (cut lengthwise along a radius of the stem), and (c) a **longitudinal-tangential** section (cut lengthwise in a tangential direction). Examine the Douglas Fir preparations first and the Scots Pine preparations

afterwards. All the sections have been cut with the microtome, stained, and permanently mounted in Canada balsam.

(a) In the **transverse section** of the Douglas-Fir wood, make out the broad annual rings. Notice the two bands of cells in each ring, namely, **spring and summer wood**, consisting of tracheides which are relatively thin-walled and wide in the radial direction, and **autumn wood**, consisting of tracheides which are relatively thick-walled and compressed in the radial direction. The **autumn wood** is much **more deeply stained** than the spring and summer wood. Notice, further, how sharp is the contrast between the dense autumn wood of one year and the more open spring wood of the next year.

Notice that **the tracheides are arranged in radial rows**. This arrangement is due to the manner in which the cambium grows and produces new wood cells. The tracheides in any row are produced by cambial action in succession, the oldest tracheide being the one nearest to the pith and the youngest one the one nearest to the cambium.

Notice also the **very narrow medullary rays**. These run radially outwards, thus passing through the annual rings of wood at right angles. Each medullary ray is only one layer of cells wide. There are about six rows of tracheides between any two adjacent medullary rays.

Resin ducts are comparatively few. Here and there in the wood you may find what appears to be a hole. Here a resin duct was situated, but its secretory cells are no longer present in the section.

(b) In the **longitudinal-radial section** the tracheides are seen in lateral view. Several annual rings are included. The spring and summer tracheides are lighter in colour and wider than the autumn tracheides. Every tracheide is a much elongated cell, pointed at each end, dead, devoid of any contents but water, and with its radial cell-walls (those now in view) exhibiting **bordered pits**. Try to find the pointed ends of some of the tracheides, and notice the **row of bordered pits** on the radial wall of each tracheide.

Each **bordered pit**, as seen in longitudinal-radial section, has a small **mouth**, the edge of which is marked by an inner circle, and a **border**, a circular ledge of cell-wall which projects slightly and extends toward the mouth, the edge of which it forms. The outer limit of the border is indicated by the outer circle and the

inner limit by the inner circle. Proceeding into the pit through the mouth, the enclosed space there present widens conically and ends against a **closing membrane** which has a diameter exactly equal to that of the border. The closing membrane is a thin part of the wall, formed from the middle lamella, which separates one bordered pit on one side of it from an exactly similar bordered pit on the other side of it. Only a small central part of a closing membrane can be seen through the mouth of a pit. For a fuller explanation of the structure of a bordered pit, the student is referred to the blackboard drawing.

Medullary rays may be seen crossing the annual rings at intervals. Each one is only a few cells high.

(c) In the **longitudinal-tangential section** the medullary rays are seen in cross-section, and the bordered pits in the tracheides are no longer seen in face view but in section on the side walls. Notice the small height of the medullary rays and their great number.

Compare the Scots Pine sections with those just previously examined. In the transverse section of the wood of Scots Pine there are many resin ducts, but they are now rather distorted and do not well show their central chamber lined by secretory cells. In the longitudinal-tangential section of the Scots Pine the short medullary rays can be seen very clearly.

Make a large-scale drawing showing the tracheides and medullary rays in the cross-section of Douglas Fir wood. Let the drawing include the **junction** of the autumn tracheides of one annual ring with the spring tracheides of the next ring. The drawing should be about three inches wide and four inches high, and the width of each tracheide should be about a quarter of an inch. As usual, indicate what the drawing is and the names of each part.

Make another drawing, showing some tracheides with bordered pits as seen in a longitudinal-radial section of Douglas Fir wood. A medullary ray may also be indicated.

Make a third drawing, if time permits, showing the appearance of the tracheides and medullary rays in a longitudinal-tangential section of Scots Pine wood.

CHAPTER XIX

A BLUE-GREEN MOULD. CORK AND BARK

Materials and preparations supplied :

- (1) A bread culture of *Penicillium glaucum* in a small crystallising dish, 4 days old ;
- (2) A hanging-drop culture of *Penicillium glaucum* made in a van-Tieghem cell, 48 hours old ;
- (3) A living twig of a dicotyledonous tree, for observations on cork and lenticels ;
- (4) A permanently mounted and stained cross-section of a one-year old stem of the Elder, *Sambucus nigra*, for observations on cork and lenticels ;
- (5) A piece of Birch-bark ;
- (6) A large piece of Cork-Oak bark, to be passed around ;
- (7) A large bottle cork, for examination of its exterior ;
- (8) A flat strip of cork, for observation of lenticels ;
- (9) A small cork, for section-cutting with a razor ;
- (10) A thick piece of true bark, for examination of its structure.

A. *PENICILLIUM GLAUCUM*

General remarks.—*Penicillium glaucum*, a Blue-green Mould, is one of the commonest of all fungi. It occurs as a green incrustation on all sorts of organic substances, e.g. rotting apples, oranges, and lemons, bread kept for some time in a damp box, old tea-leaves left in a tea-pot, old yeast cultures (at the surface), and leather boots kept in a damp cupboard. Closely related species give the green colour to the veins of certain cheeses, e.g. *Penicillium Roquefort* in Roquefort cheese. The presence of a blue-green mould in such cheeses as Roquefort, Gorgonzola, and Stilton is by no means a sign of unsound composition, for the mould consumes the acid produced by the lactic-acid bacteria, thus preventing the acid from retarding the action of the albumin-degrading bacteria and thereby assisting in the process of cheese-ripening.

Penicillium glaucum, like all other fungi, excepting the Yeast plant, when fully developed has two parts : (1) a vegetative and (2) a reproductive.

The vegetative part of a *Penicillium* plant, like that of a Pin Mould or a Mushroom fungus, is called the **mycelium**, while the reproductive part consists of **conidiophores** and **conidia**. A **conidiophore** is an aerial hypha which bears conidia, and a **conidium** is a spore which is not contained in a sporangium but which is constricted off the end of a hypha. The conidia are dispersed by the wind.

(1) The **mycelium** of *Penicillium glaucum* originates from a spore and is made up of numerous much-branched hyphae which are distinctly smaller than those of the Pin Mould or the Bread Mould. Whereas in the Pin Mould and Bread Mould the main hyphae are much thicker than the lateral branches, in *Penicillium* the main hyphae and the branch hyphae are all of almost uniform thickness. Branching of the mycelium takes place **monopodially**, i.e. a new branch grows out from the side of an apical cell or just behind the cross-wall which bounds the front of an ordinary cell. The growth of each hypha in length is **apical**. Some of the hyphae of a *Penicillium* mycelium grow over the surface of the organic substratum (bread, apples, etc.), while others penetrate into its interior and ramify there in all directions. A Blue-green Mould, like the Pin Mould or the Bread Mould, acts upon the organic matter upon which it lives by means of **enzymes** which it excretes through its cell-walls and then, through its cell-walls, it absorbs the products of enzymatic digestion. Among the enzymes produced by *Penicillium* are: (1) **diastase**, which turns starch into sugar, (2) **lipase**, which hydrolyses fats and oils, decomposing them into glycerine and fatty acids, (3) **emulsin**, which decomposes glycosides (e.g. amygdalin into benzoic acid, prussic acid, and glucose), and (4) a **protease**, which breaks down certain solid proteins into a simpler soluble form known as peptone.

The mycelium of *Penicillium glaucum* and of other Blue-green Moulds differs from that of a Pin Mould or Bread Mould in being **multicellular** instead of unicellular, thus exhibiting a more advanced state of organisation. The mycelium is **septate**, i.e. its hyphae have **septa** (sing. *septum*) or cross-walls at intervals which divide up the whole mycelium and also the sporophores into elongated cylindrical cells. Each cell differs from a cell of the Flowering Plants in that it **contains a number of very small nuclei** instead of a single nucleus. The nuclei in living mycelia are always too small and inconspicuous to be seen with the

microscope. Only by fixing and carefully staining the hyphae can the nuclei be brought into view.

(2) **The conidiophores** of *Penicillium glaucum* are numerous hyphae which grow out from the mycelium into the air so as to project more or less perpendicularly from the substratum. Each conidiophore, at a short distance from the substratum, branches and rebranches several times, and then forms numerous chains of green conidia, one chain terminating each of the ultimate branches of the conidiophore. The branches at the end of a conidiophore along with their chains of conidia diverge from one another something like a pencil of diverging light rays. Hence the generic name **Penicillium**. There are 7-10 or more conidia in each conidial chain, and every conidium has a **bluish-green cell-wall**. Hence the specific name **glaucum**. The blue-green pigment is in no way related to chlorophyll and must not be confused with it. It is the numerous chains of blue-green conidia at the ends of the closely-set conidiophores which give the blue-green colour to a Blue-green Mould when this is seen on mouldy bread, fruit, etc., with the naked eye.

Examination of a Bread Culture of *Penicillium glaucum*.—The piece of bread in the dish was moistened with sterilised water and *Penicillium* spores were sown upon it four days ago. The spores germinated within a few hours and produced mycelia. The mycelium ramified over and in the substratum and, within 24 hours, the surface hyphae began to produce numerous aerial conidiophores, and these soon branched at their ends and began to constrict off their chains of green conidia. It is the vast number of these chains of minute green spores which give the mould its blue-green colour. As already stated, the blue-green pigment is situated in the cell-walls of the conidia and chemically is quite different from chlorophyll.

With a needle and knife, remove from the bread a very tiny bit of the fungus, place it in a drop of water on a slide, tease it out somewhat, add a cover-glass, and observe with the microscope. When placed in water, the chains of conidia readily separate into their constituent parts, and you will therefore find in your preparation numerous **isolated green conidia**. The **conidiophores** and some of the **mycelium** will be also present in the preparation, but such a preparation as this is not favourable for observing them owing to their dislocation and to small masses of air held between the conidiophores and spores.

Examination of a Hanging-drop Culture of *Penicillium glaucum*.

The mode of making a hanging-drop culture of a mould in a closed glass-ring damp-chamber (a van-Tieghem cell) was explained in Chapter XVIII. The hanging drop consists of the same kind of nutrient gelatine as was used for the Pin Mould. Some *Penicillium* spores were sown in the drop two days ago. They germinated and produced a branching mycelium, and the mycelium is now giving rise to conidiophores and conidia.

With the **low-power** objective examine the hanging-drop culture. Observe (1) the **mycelium** growing in the nutrient gelatine and over the moist surface of the cover-glass, and (2) the **conidiophores**, growing downwards from the hanging drop into the air of the chamber and bearing chains of **conidia** at their ends.

In a hanging-drop culture, one sees all the **different parts of the mould undisturbed** and as they are in nature. Hence the advantage of making hanging-drop cultures for the study of the life-history of *Penicillium* and of fungi in general.

With the **high-power** objective you should be able to examine the details of structure of the mycelium. Notice that the **hyphae** are all very **thin** and more or less **equal in diameter**, that the mycelium is much **branched**, and that the branching is not forked but **monopodial**. With care you may be able to observe some of the **septa** which divide the mycelium up into its component cells.

Since the conidiophores project downwards so far below the cover-glass, it may not be possible to focus on any of their ends with the **high-power** objective without smashing the cover-glass. If you want to see the chains of conidia with the high-power, the best way to proceed is as follows: With the **low-power** objective find the conidiophore which has its end nearest to the cover-glass. Then turn on the **high-power**, and try to focus the desired object. It may be that this is possible. If you keep the left hand on the preparation and keep moving it very slightly you can feel when the high-power comes into contact with the cover-glass. When contact has been made, further screwing downwards can only smash the cover-glass without bringing the object into focus.

Make drawings of : (1) part of a **mycelium** showing hyphal branches and septa (if you have observed them), (2) one or two **conidiophores** springing from the mycelium (this represented semi-diagrammatically) with branches at their ends bearing **chains**

of **conidia**, and (3) some isolated **spores**. A single conidiophore should be drawn 3–4 inches high, and then all other parts of your drawing should be made on the same scale. As usual, indicate what your drawing is and the names of its parts.

B. GENERAL REMARKS ON CORK AND LENTICELS

The **epidermis**, as already observed by you, is one of the **primary tissues**, for it is always present in very young stems, where it covers and protects the cortex and all the other internal tissues. However, in dicotyledonous shrubs and trees, as a stem increases in thickness, the **epidermis** sooner or later **dies** and its place is taken by a stronger **secondary tegumentary tissue** known as **cork**.

Cork is always developed from a special **cork-cambium** (phellogen). Cork-cambium, which is quite distinct from the cambium which forms secondary xylem and phloem, as a rule comes into existence as follows. The outermost layer of the cells of the cortex, i.e. the layer just under the epidermis, becomes meristomatic. Two tangential cell-walls are formed across each of these cortical cells, and the middle cell of the three thus brought into existence becomes an **initial cork-cambium cell**. By the stretching of the cork-cambium cells in the radial direction and by their division with the formation of tangential cell-walls, a layer of cells is gradually cut off on the **outside** of the cork-cambium. It is this layer of cells which is known as **cork** (phellem). In some stems the cork-cambium cuts off not only cells on its outside, which become a layer of cork, but also a certain number of cells on its inside, these inner cells forming a layer of chlorophyll-bearing parenchyma which is added to the primary cortex and may be called **cork-cortex** (phelloderm). It is convenient to use the term **periderm** to include the cork-cambium and all its products, i.e. both cork and cork-cortex.

Summary of Cork Tissues.

- | | |
|------------------------------------|--------------------|
| A. Cork (phellem) | } Periderm. |
| B. Cork-cambium (phellogen) | |
| C. Cork-cortex (phelloderm) | |

The **cork cells** produced on the exterior of a cork-cambium have special characteristics of their own. They are **tabular** in shape, fit closely together **without any intercellular spaces**,

and their cell-walls do not consist of pure cellulose but contain a fatty substance called **suberin** and are said to be **suberised**. This **suberisation** of the cell-walls of cork cells, together with the absence of any intercellular spaces except at the lenticels, endows cork with the property of being very **resistant to the passage of water** into it or through it, and it is this impermeability to water which makes cork of so much value for stopping bottles, etc.

Cork cells usually have rather thin walls and, when fully formed, they **die**. After their death they lose their protoplasm and cell-sap, these cell-contents becoming replaced by **air** often accompanied by a yellow or brown substance. Since cork cells contain air and their walls are impermeable to water, **cork will float** in water for an indefinite time. Hence its use for life-belts, etc. The air-content of the cells makes cork a **bad conductor of heat**. Hence cork is used for mats on which to set hot tea-pots, etc., and for stepping-boards in bathrooms.

The **light-brown colour** of the **cork of the Cork Oak** used for making bottle stoppers is due to the **colour of the cell-walls** only, but the **deep-brown** or **blackish** colour of the thin cork layer of many **twigs**, e.g. Basswood, is due in part possibly to the brown cell-walls but chiefly to the **brown cell-contents**.

Cork is a very tough yet distinctly elastic secondary tissue, it resists the passage of water vapour through it except at the lenticels, and it is very resistant to penetration by the hyphae of most fungi. It is therefore **admirably fitted to replace the epidermis** and to protect twigs and branches from deleterious external influences.

Lenticels.—In dicotyledonous shrubs and trees, certain **pores** in the cork, called **lenticels**, make their appearance simultaneously with the formation of the layer of cork under the epidermis. The cork-cambium (phellogen) here and there below a stoma does not form ordinary cork cells on its exterior ; but, instead, it gives rise to a special **lenticel tissue** composed of so-called **complementary cells** which are more or less rounded off and are traversed by **intercellular spaces**. These cells press the epidermis outwards and eventually cause its rupture. On its inner side the cork-cambium (phellogen) of a lenticel regularly forms a layer of **cork-cortex** (phelloderm).

Summary of Lenticel Tissues.

- A. **Lenticel tissue**, composed of **complementary cells** with intercellular spaces between them in the radial direction.
- B. **Cork-cambium** (phellogen) of the lenticel, in lateral continuity with the ordinary cork-cambium between the lenticels.
- C. **Cork-cortex** (phelloderm), added to the primary cortex.

Lenticels can be readily seen with the naked eye on the exterior of twigs, branches, and woody roots of shrubs and trees. Their **function** is to maintain the **gaseous exchange** between the outside air and the intercellular spaces of the plant. In the process of **respiration**, which goes on night and day in every living cell of a stem, the lenticels permit of a passage of oxygen into the plant and of carbon dioxide out of the plant.

C. THE LAYER OF CORK IN A TWIG

Examination of the Exterior Surface of a Twig.—Examine the twig provided. Note its **dark colour**. This is due to a very **thin layer of cork** which lies just beneath the epidermis. Twigs, when newly formed in the spring, are **green**, owing to the presence of chloroplasts in the cortex ; and, later in the year, they become **dark brown** or **blackish**, only as a result of the formation of a **sub-epidermal cork layer**.

Look for lenticels. You should find these curious pores scattered here and there in every twig. What the stomata are to the leaf, the lenticels are to a twig or branch, i.e. structures which permit of gaseous exchange between the outside and inside of a plant.

Make a sketch showing a twig. Indicate its **lenticels** and the **cork layer** between the lenticels.

Examination of Cork as seen in a Transverse Section of an Elder Twig.—A permanent stained transverse section of a one-year-old twig of *Sambucus nigra*, the Elder of England, is provided. Examine it first with the low-power objective and then with the high. Our business just now is to study in detail the layer of cork just beneath the epidermis ; but, before doing this, we may take a **rapid survey** of the **various tissues** of which the stem is composed. These tissues in the centripetal direction may be summarised as follows :

Summary of Tissues seen in a Transverse Section of a One-year-old Elder Twig.

1. **Epidermis** one layer of cells thick.
2. **Cork** a layer of tabular cells about 4 cells deep, arranged in rows, just under the epidermis.
3. **Cork-cambium** one layer of cells thick, made up of flattened cells from which the cork layer has been formed.
4. **Cortex**(a) the outer cortex, composed of smaller cells with thickened walls (collenchyma).
(b) the inner cortex, composed of larger cells with walls not thickened (parenchyma).
5. **Phloem** a thin ring of cells with some thick-walled bast fibres on its exterior, the other cells being sieve-tubes, companion cells, and bast parenchymatous cells.
6. **Cambium** a thin very distinct layer of flattened thin-walled cells between the secondary phloem layer and the secondary xylem.
7. **Xylem**(a) secondary xylem, the first annual ring of wood, containing large scattered pitted ducts (wood vessels) and smaller thick-walled wood fibres, wood parenchymatous cells, etc.
(b) primary xylem, wedge-shaped xylem tissue-masses projecting into the pith at intervals around its margin.
8. **Pith** a central mass of parenchymatous cells. Certain very darkly stained cells in it are **tannin sacs**, i.e. cells containing tannin.

Special Study of the Cork and its Lenticels.—With the low-power objective look all around the **layer of cork** and notice that in one or two places it is interrupted by **lenticels**.

Observe that the **epidermis** is still present on the exterior of the

twig where there are no lenticels, but that at a lenticel it has been ruptured and pushed outwards.

Notice that, where there is no lenticel present, the layer of cork is about four cells deep, that the cork cells are in **radial rows**, that there is a **cork-cambium cell** (flattened in the radial direction) at the base of each row, and that the tissue interior to the cork-cambium is the primary cortex. There is **no cork-cortex** in the Elder, except at a lenticel. The order of the tissues from without inwards, therefore, is: (1) epidermis, (2) a layer of cork, (3) cork-cambium, and (4) primary cortex.

Notice further, that the cork cells have rather **thin** walls and relatively **large cell-lumina**, and that between adjacent cork-cells there are **no intercellular spaces**.

Now examine a lenticel. Notice that the ordinary cork-cambium and the cork-cambium of the lenticel are continuous with one another, and that outside the cork-cambium of the lenticel there is a mass of **lenticel tissue** composed of **complementary cells** which have pressed against the epidermis and caused its rupture. Inside the cambium and formed by it is a certain amount of **cork-cortex**. Look for **intercellular spaces** running radially in the lenticel tissue and cork-cambium, and remember that it is these spaces which are of so much importance in the functioning of a lenticel.

Make a drawing to illustrate the **external tissues** of the twig of an Elder as seen in your section. Show: (1) the **epidermis** and how it is ruptured at a lenticel, (2) an ordinary **layer of cork**, (3) the **cork-cambium**, (4) a few outer cells of the **primary cortex**, and (5) a **lenticel**. In the lenticel show the **lenticel tissue**, composed of **complementary cells**, the **cork-cambium of the lenticel**, and the **cork-cortex**, abutting on the outer primary cortex. The epidermis may be drawn first, and each of its cells should be made from one-quarter to one-third of an inch wide. Then everything else should be drawn in proportion. Label the parts of your drawing.

D. THE CORK OF THE BIRCH

So-called **Birch-bark**, obtained from the Paper Birch, *Betula alba* var. *papyrifera*, is in reality a **layer of cork** which is added to each year through the action of a cork-cambium. Each **annual ring or layer of cork** is exceedingly **thin** and **separable** from its fellows, and it is owing to Birch-bark being composed

of many successive and separable layers of cork that it is possible to split it up into very thin papery sheets.

As the trunk of the Birch tree grows older and thicker, the outer and older layers of cork become too small to envelop the tree trunk, in consequence of which they snap and pull off in papery fragments ; while, at the same time, newer and larger annual layers of cork are being added by the cork-cambium each year to the inside of the whole cork layer (the Birch-bark).

Notice the **lenticels** scattered in the piece of Birch-bark provided. The lenticels are at first quite small and more or less rounded like those already seen in a thin twig. In the Birch they **grow wider as the tree increases in thickness**, so that the widest lenticels are on the thickest trees. This widening of the lenticels with age can also be well observed in the trunks of Cherry trees. Observe that the lenticels are continuous through the Birch-bark from one side to the other. Observe further, that the brown lenticel tissue which occupies the lenticels is of very different texture from the ordinary cork tissue. In a lenticel the cells are always rounded off at their corners and loosely packed together, so that the lenticel constitutes a porous channel suited for gaseous interchange.

Birch-bark **burns well** because the cork cells contain a substance known as **birch-resin**, and it is this resin that gives to Birch-bark its peculiar white colour.

Draw a small piece of Birch-bark in surface view natural size, and show three or four transversely elongated lenticels.

E. THE CORK OF THE CORK OAK

The cork used for making stoppers for bottles, etc., is obtained from *Quercus suber*, the **Cork Oak**, and it is all produced by the action of a cork-cambium. The Cork Oak grows in countries around the Mediterranean, and much cork is produced in Spain. The bark is first taken off a tree when it is fifteen years old ; but the cork is of poor quality. It takes about ten years to grow again and is then cut a second time. This crop is worth more than the first, but is still not very valuable. After this, the cork is cut from the tree every eight or ten years and it grows better after each cutting. It is said that a tree thus barked will live for 150 years. In removing the cork which is composed of dead

cells from a tree, care is taken not to injure the living phloem and cambium ; for, if these tissues were destroyed, no new cork-cambium could be formed and the tree would die.

The cork of the Cork Oak exhibits **annual rings of growth**. Each ring consists of two layers : (1) a broad layer of soft large cells produced in the spring and summer and (2) a very thin layer of darker brown, radially narrower cells produced in the autumn. These layers alternating with one another in successive annual cork rings can be readily seen in bottle cork.

Examination of Bottle Cork.—A large bottle cork is provided. Please **do not damage it**, as it is required for future classes, and a smaller cork is at your disposal for section-cutting. Find the **annual layers of cork**, and notice that in each ring the autumn cork consists of a narrow layer of browner cells. **How many years** did it take to grow the layer of cork included in your bottle cork ? Record the answer in your book.

Now observe the **lenticels**. These run through the annual rings of cork at right angles. This might have been expected, since in a Cork Oak all the lenticels run in a **radial direction**. Notice how brown and relatively loose the lenticel tissue in a lenticel is. Even when the cork surrounding a living Cork Oak has attained a thickness of two inches, the **lenticels** still form **continuous passages** through it, thus providing for the gaseous interchange with the outer atmosphere required for respiration by the living cells in the tree trunk.

Notice how the bottle cork has been cut by the manufacturer out of a piece of Cork Oak bark—in such a way that the **lenticels run from side to side** in the cork and **not up and down**. If in a bottle cork the lenticels had an up and down direction, the cork might well be suited for a salt bottle (very wide corks for salt bottles are in fact so made), but it would be unsuitable for a bottle containing a liquid ; for, if the bottle were turned on its side, some of the liquid might slowly escape through the lenticels.

Make a drawing of a tilted bottle cork, natural size, so as to show a top and a side view at one and the same time. Indicate the **annual rings of cork** and the **lenticels**. Hold the cork so that the annual rings at the top are parallel to the top of your page.

Examination of a Piece of Sheet Cork.—The strip of cork provided was cut out of a large sheet of cork in a tangential

direction. Hence the lenticels are seen in transverse section. Notice how very numerous the lenticels are.

To Make a Microscopic Preparation of Cork.—Take the smaller bottle cork provided and, with a dry razor, cut one or two very thin **transverse sections** from the top. Mount them in a drop of water under a cover-glass. Examine with the microscope.

Observe that the **cork cells** have **thin walls** and look dark owing to their lumina being **filled with air**. Notice, further, that the **cells are all in rows** (a fact which is readily explicable when one remembers that the cork was formed by a cork-cambium), and that between the cork cells there are **no inter-cellular spaces**.

At the edge of your section you will doubtless be able to find some cells which were cut open by the razor and in which, therefore, **water has replaced the air in their lumina**. Such cells can more readily be examined in detail than those which are intact and filled with air.

Make a drawing showing a few **rows of cork cells** fitting together without any intercellular spaces between them. Indicate the suberised **cell-walls** and the **air** in the cell-lumina. Use your own judgment as to the scale of the drawing.

Microchemical Reactions of Suberised Cell-walls.—The suberised membranes of cork cells behave toward certain chemical reagents in a very different way to cellulose. Thus, whereas pure cellulose, e.g. in the wall of a Cotton hair, turns violet with chlor-zinc iodine and is dissolved by strong sulphuric acid, the **suberised walls of cork cells turn yellow with iodine** and are **not dissolved by strong sulphuric acid**. This remarkable resistance to sulphuric acid is also shown by the **cuticle**.

F. THE STRUCTURE OF BARK

True bark, such as one observes on the tree trunk of an Elm, Oak, or Pine, is not a single layer of cork but something more. It is a **series of thin, somewhat lenticular layers of cork** which have been formed from a series of cork-cambiums produced in centripetal succession in the deeper cortex and in the secondary phloem, together with **islands of cortex and older secondary phloem** enclosed between them. Bark, in many trees, becomes **scaly** and each **scale** (except the outermost ones which have originated in the cortex) is a lenticular or plate-like mass of

secondary phloem bounded in front and behind by a thin layer of cork. The order of the tissues in the centripetal direction in a living Oak or Elm tree trunk may be summarised as follows :

**Summary of Tissues in a Tree Trunk of an Oak,
Elm, or Pine.**

1. **Bark** a thick mass of dead tissues consisting of successively deeper thin layers of cork with islands of cortex and older inactive phloem in between them.
2. **Active phloem** the newer inner phloem, a thin layer, in which the cells are all living and the sieve-tubes conduct food materials.
3. **Cambium** between the thin layer of active phloem and the xylem.
4. **Xylem** the annual rings of wood and the primary xylem.
5. **Pith** a small core of parenchymatous cells.

All the tissues of which bark is composed (cork layers, islands of cortex and phloem) are **dead**. The **outer part of the bark** becomes too tight for the growing tree trunk and it therefore becomes **ruptured vertically**, thus giving rise to the **vertical clefts** everybody may observe in the bark of such trees as the Oak and Elm ; but the **inner part of the bark never ruptures completely**, owing to the fact that, with the activity of each new cork-cambium, it becomes larger and larger. We may conclude, therefore, that an Oak or an Elm tree provides itself with a tough, close-fitting, dead garment, namely, the bark, which while necessarily fissured on its outside is never broken through and which, therefore, very efficiently protects the delicate inner tissues (active phloem, cambium, and young wood) throughout the tree's life.

Examination of a Piece of Bark.—Examine the piece of bark. Notice its series of thin layers of cork and the relatively thick masses of phloem (phloem islands) enclosed between them.

CHAPTER XX

THE MUSHROOM. RESIN DUCTS. SIEVE-PLATES. THE
INTERNAL STRUCTURE OF A FOLIAGE LEAF

Materials and preparations supplied :

- (1) A piece of Mushroom spawn ;
- (2) A living Mushroom ;
- (3) A spore-deposit, to be passed around ;
- (4) A *Fomes pinicola* fruit-body attached to wood, to be passed around ;
- (5) A transverse section of a twig of *Pinus sylvestris*, for a study of resin ducts ;
- (6) A transverse section of a Cucumber stem, for a study of sieve-plates ;
- (7) A skeleton leaf of a Poplar ;
- (8) A transverse section of a Holly leaf ;
- (9) A transverse section of a Tradescantia leaf, to supplement (8).

A. THE MUSHROOM

Psalliota campestris, the **Common Mushroom** or the **Field Mushroom**, is a fleshy fungus often found growing amongst short grass in pastures, lawns, and open places, or in gardens on soil heavily manured with horse dung. Its fruit-bodies are edible and are much esteemed as a delicacy for the table. On this account the Mushroom plant is cultivated on a large scale in caves, greenhouses, and cellars.

Like all other fungi, the Mushroom plant lacks chlorophyll, and therefore cannot use sunlight as a source of energy to manufacture sugar from carbon dioxide and water. Not being able to carry on the work of photosynthesis, it depends for its existence on complex organic substances already manufactured by the green plants, and it finds these substances (in which much energy is stored up) in the turf of fields and in horse manure. Since the Mushroom plant, like the Yeast plant and Moulds, lives on **dead** organic matter, we can refer to it as a **saprophyte**.

There are several hundreds of species of fleshy fungi (**agarics**) to be found in woods and fields which, in their general organisation, resemble the Common Mushroom. Some generations ago,

in England, it was almost universally believed that only two kinds of these fleshy fungi were edible, namely, the **Common Mushroom** and the **Horse Mushroom** (a larger, coarser species), and these were always called **mushrooms**, while all the other species were classed together as dangerous and were popularly known as **toadstools**. It is now known that many so-called toadstools are perfectly good to eat and have pleasant and distinctive flavours of their own. Unfortunately, however, there are a few toadstools, e.g. *Amanita phalloides*, the **Deadly Amanita**, also known as the **Destroying Angel**, which are exceedingly poisonous. Every year in Europe and North America many scores of people die owing to their having eaten poisonous fungi, in particular the Deadly Amanita, in mistake for edible ones. After a person has partaken of a dish of fruit-bodies of the Deadly Amanita, no effect is noticed for some time, for the poison is slow in making itself felt. However, about 6–15 hours after the meal, there is sudden and severe pain in the abdomen. This is followed by loss of strength and of flesh and, if the amount of the fungus eaten has been sufficient, the patient gradually sinks into a profound coma and dies in from three to five days.

If one desires to eat any of the edible toadstools, it is necessary **to learn to distinguish them** from the dangerous species by their specific characteristics and to ascertain their record as determined by those who have eaten them in the past. It is popularly supposed that an edible fungus can be told from a poisonous one by means of one or other of a number of simple tests: e.g. (1) if a fungus has a cap which “peels,” it is edible; and (2) if, when being cooked, a fungus turns a silver coin or a silver spoon black, it is poisonous; but, if it does not do this, it is edible. **Not the slightest value can be placed on any one of these tests**, and hundreds of deaths have been caused by ignorant people relying upon them.

The Mushroom plant, like the Bread Mould or a Blue-green Mould, consists of two parts: (1) the **vegetative** and (2) the **reproductive**.

(1) The **vegetative** part of a Mushroom plant consists of a **mycelium** made up of much-branched **hyphae** (sing. *hypha*), most of which take their own course through the substratum, while others grow together side by side in intimate union so as to form thin white **mycelial cords** which can just be seen with the naked eye. The so-called **mushroom spawn** used in the cultivation of

mushrooms consists of mycelial hyphae and mycelial cords embedded in compressed blocks of horse and cow manure. If small pieces of these blocks (about the size of one's thumb) are set in a Mushroom bed made of fermented stable manure, the mycelium grows out into the manure and, in the course of 6-8 weeks, it may give rise to the reproductive parts of the fungus which we know as **mushrooms**. Mushrooms develop at the expense of the mycelium from which they spring, i.e. the mycelium becomes exhausted in producing them.

Examination of a Piece of Mushroom Spawn.—With the naked eye examine the piece of brick spawn provided. Note that it consists of hard dry dung containing thin white mycelial cords which can just be seen. The isolated mycelial hyphae which ramify through the dung in all directions are invisible.

(2) **The reproductive** part of a Mushroom plant consists of the fleshy organs which are sold and eaten under the name of **mushrooms**. These are the fruiting parts of the Mushroom plant and may be referred to as **fruit-bodies**. They are produced aerially and their one great function is to produce and liberate many millions of minute **spores** (unicellular reproductive bodies). In more technical language, a mushroom must therefore be called a **sporophore** (spore-bearer). As a sporophore, a mushroom is much more complicated than the sporophore of a Blue-green Mould. In a **Blue-green Mould** a **sporophore** consists of a **single hypha**, whereas in a **Mushroom plant** a **sporophore** consists of **thousands of hyphae combined together** to form a thick stalk and cap. Hence we are justified in considering a mushroom as a **compound sporophore**.

Examination of the Sporophore of a Mushroom Plant.—Notice that the sporophore (mushroom) provided is a very massive organ, apparently quite different in structure from the filamentous mycelium upon which it was developed. However, it is made up of large numbers of hyphae resembling those of the mycelium but attached to one another so as to form a compact mass of fungal tissue. This tissue is sometimes said to be a **false tissue** since, in its origin, it differs so profoundly from the tissues of the Flowering Plants.

Observe that the sporophore as a whole is **umbrella-shaped** and that its two chief parts are (1) the **stipe** (stalk) and (2) the **pileus** (cap). The pileus produces and liberates the spores on its under side, and the stipe raises the pileus to such a height

above the ground that the spores, when falling from the pileus, can be readily carried off by the wind.

The **stipe** is a stout structure, well fitted to support the massive pileus. Notice, if the pileus has expanded, that the stipe is encircled by a somewhat torn membranaceous ring known as the **annulus** (ring). This, when the mushroom was younger and in the so-called **button stage**, formed part of a thin white membrane, the **veil** (velum partiale), which extended from the edge of the pileus to the stipe and closed the so-called **gill-chamber** ; but, when the pileus expanded, the veil became torn peripherally, with the result that some of it was left hanging in fragments around the edge of the pileus while the rest of it was left attached to the stipe as an annulus or ring.

The **pileus** will be seen to consist of two parts : (1) the **pileus-flesh** and (2) the **gills** or **lamellae**. The **pileus-flesh** supports the gills and holds them fixed under normal conditions in vertical planes. The **gills** are numerous thin brown plates, which are all **radially arranged** between the stipe and the edge of the pileus. They owe their name to the fact that they have a superficial resemblance to the gills or branchial lamellae of certain fishes. Two long gills, in passing from the stipe to the edge of the pileus, diverge from one another. Notice how the space between them is filled up in part by the addition of shorter and still shorter gills. Each individual gill is shaped like the blade of a pen-knife with the cutting edge directed downwards toward the ground. Under normal conditions, adjacent gills do not touch one another but, between every two, there is an **interlamellar space**. Notice that there are as many interlamellar spaces as there are gills. These spaces, although so narrow, are of great importance for the escape of the spores.

The under side of the pileus is covered by a **spore-bearing layer** of hyphal cells known as the **hymenium**, and this forms the outermost layer of cells of each gill. The gills, as it were, throw the hymenial layer into folds, thus greatly increasing its area without any serious loss of compactness to the sporophore as a whole. The gills, through the activity of their hymenium, produce and liberate millions of microscopic **spores**. If the cap of a mushroom is cut off, laid on a sheet of white paper, covered with a bell-jar and left undisturbed overnight, in the morning one finds that the spores liberated by the gills have settled on the paper in vast numbers and have there formed a chocolate

spore-deposit. The spores in the deposit are heaped up in radial lines corresponding to the interlamellar spaces of the gills, and on this account a spore-deposit is often referred to as a **spore-print of the gills.**

Examine the spore-deposit passed around to you for your inspection. Note its colour, which is due to the colour of the spores, and the radial lines corresponding to the arrangement of the gills. In one spore-deposit, made by a 3-inch-wide pileus of a wild Mushroom in 48 hours, there were about 1,800,000,000 spores, so that, on the average, forty million spores must have fallen from the gills each hour or over half a million a minute.

Cut a sporophore vertically down the middle into two equal halves and **make a drawing** of one of the surfaces thus exposed. Indicate: the **stipe** and its **annulus**, and the **pileus** with its **pileus-flesh** and **gills** (lamellae).

Examination of some Hyphae from a Stipe.—With a needle or knife remove a tiny bit of the soft substance of the **central** part of the stipe, place it in a drop of water on a slide, tease it out with two needles or a needle and knife, add a cover-glass, and examine with the microscope.

Notice that the hyphae are elongated cylindrical structures with cross-walls (septa) in them at intervals. The hyphae, here and there, are branched or attached to one another laterally. In some of the cells of which the hyphae are made up you may be able to observe cytoplasm and possibly vacuoles, but the nuclei cannot be seen without special staining to bring them out.

To make a Transverse Section through some Gills.—Lay the pileus on its back on the table and with a knife cut out from it a piece of pileus about 1 cm. square, the piece to include both flesh and undamaged gills. Some of the flesh may be trimmed off. Hold the piece of material in the left hand with the flesh away from you and the gills pointing toward you; and then, with a razor wetted with water, cut some thin **transverse** sections through some of the gills and the flesh which holds them together. Mount some of the best sections in a drop of water, add a cover-glass, and examine with the microscope.

Examination of the Preparation.—Notice with the low-power objective that each gill in cross-section is **wedge-shaped** and that there are many isolated purplish-brown spores scattered about.

Each gill will be seen to have three chief parts: (1) the **trama**, a central core of elongated hyphae; (2) the **subhymenium**, a

layer of somewhat rounded cells outside the trama and under the hymenium ; and, finally, (3) the **hymenium**, the external layer of cells which produces the spores on its exterior.

The **hymenium**, or spore-bearing layer of the gill, is made up of somewhat club-shaped cells which stand side by side with their long axes perpendicular to the surface of the gills. The cells of the hymenium are of two kinds: (1) sterile **paraphyses** which never produce any spores, and (2) **basidia** which produce the spores (**basidiospores**).

Look for basidia. A full-grown basidium is an elongated cell which projects slightly beyond the other cells of the hymenium and consists of three parts: (1) a **basidium-body** which is club-shaped; (2) two little slenderly conical stalks or **sterigmata** (sing. *sterigma*); and (3) two **spores** or **basidiospores**, each one borne on the end of a sterigma. While in **cultivated mushrooms** each basidium has only **two** sterigmata and **two** spores, in **wild mushrooms** and most other agarics each basidium has **four** sterigmata and **four** spores. There are many millions of basidia on the gills of a mushroom, but they do not all come to perfection together. They produce their sterigmata and spores in succession and so spores are liberated from a mushroom in a continuous stream for some 5-6 days. In your preparation some of the basidia will be seen to bear **small, rounded, colourless spores**. These spores are **immature**. It would have taken some hours for them to attain full size and to develop their pigment. In making the preparation, unfortunately, the ripest spores came away from their sterigmata into the water, so that it is almost impossible to observe fully ripe spores still attached to the basidia.

In nature, when a spore is ripe, its basidium shoots it away from the sterigma into an interlamellar space where it falls down slowly between the gills (rate of fall about 1 mm. per second). On escaping from the pileus, every spore is carried off by the wind. If a spore finds its way into a suitable substratum (turf, horse manure, etc.), it germinates and produces a mycelium which in turn may produce new sporophores.

Make a drawing showing part of a gill in cross-section. Indicate: (1) the **hymenium** with its **basidia**, some without spores and some with; (2) the **subhymenium**, made up of rounded cells; and (3) the **trama**, the central elongated septate hyphae. In a mature basidium indicate the **basidium-body**, the two **sterigmata** (sing. *sterigma*), and the two **basidiospores**.

In the **wood-destroying fungi** which attack living or dead trees, the **mycelium** feeds upon the wood (the hyphae penetrate through the cell-walls), while the **fruit-bodies** (sporophores) are produced as **brackets** on the bark. On the under side of these brackets, instead of gills there are exceedingly **fine tubes**, shaped like inverted test-tubes. These **hymenial tubes** are lined internally with the hymenium. This **hymenium** bears **basidia** which shoot their **spores** into the tubes down which they fall to escape from the fruit-body and be carried off by the wind. A specimen of a wood-destroying fungus may be shown to you.

B. RESIN DUCTS

Resin ducts or **resin passages** are found in many of the Coniferae, e.g. *Pinus Banksiana*, the Jack Pine, and *Abies balsamea*, the Balsam Fir. A preparation is provided in which you may observe their structure with the microscope.

Examine the section provided. It is a cross-section of a twig of *Pinus sylvestris*, the Scots Pine. The twig when gathered was two and a half years old, as is shown by the rings of wood. From without inwards its tissues may be summarised as follows :

Summary of Tissues in a Two-and-a-half-year-old Twig of *Pinus sylvestris*.

1. **Epidermis** in traces, broken up, stained blue.
2. **Cork layer** a yellowish layer with a central row of thick-walled cells and a cork-cambium below.
3. **Cortex** a thick layer of parenchymatous cells, containing a few **large resin ducts**.
4. **Phloem** a fairly thick layer made up of rows of radially compressed sieve-tubes, larger bast parenchymatous cells, and medullary rays.
5. **Cambium** a layer of thin-walled cells between the phloem and the xylem.
6. **Xylem** two and one-half rings of secondary xylem containing a number of **small resin ducts**, and some primary xylem projecting into the pith.
7. **Pith** a large central core of parenchymatous cells.

Our business just now is not to study the above tissues in detail but to confine our attention chiefly to the **resin ducts**. These, as shown in the above summary, are present in the **cortex** and in the **secondary xylem**.

Notice that the **resin ducts in the cortex** are much **larger than those in the wood**. Observe that each resin duct is lined by an **epithelial layer** of thin-walled cells which bulge out into the central canal. These cells are **glandular** : they produce a **very sticky resinous secretion** and pour it into the resin passage from which it may flow out when the tree is wounded. The resin ducts are connected together into a system by horizontal resin ducts which run radially through certain of the medullary rays, but your section is not likely to show these connections. In a cortical resin duct the number of glandular cells in view is about 10-20, while in a resin duct in the wood the number is limited to about 4-5.

Draw two resin ducts, one from the cortex and the other from the **xylem**. Show the central **resin passage**, the lining layer of **epithelial cells** (glandular cells) and some of the cells surrounding the resin duct. The cortical resin duct should have an external diameter of an inch or more, and the xylem resin duct should be drawn on the same scale.

C. SIEVE-PLATES

A sieve-tube is made up of a series of elongated cells and the partition walls or **septa** dividing one cell from another are known as **sieve-plates**. Each sieve-plate is perforated by a number of small holes. Hence its name. A preparation is provided in which you may observe sieve-plates without difficulty. It is a transverse section of the stem of a Cucumber plant, *Cucumis sativa*. The Cucumber plant is a climber which attaches itself to shrubs and trees by means of its very sensitive tendrils. It is known that in climbing plants in general the sieve-tubes and also the wood vessels have a very large diameter. It is on account of the very large size of its sieve-tubes that the Cucumber plant has been chosen for the study of sieve-plates.

Examine the Cucumber section provided. Look at it first with the naked eye against a white background and then you will perceive that it contains an irregular ring of about eight vascular bundles, three of which are nearer the centre and five farther out.

With the low-power objective one can make out the following tissues :

Summary of Tissues in a Cucumber Stem.

- I. **Epidermis** one layer of cells.
- II. **Cortex** a rather thin layer of cells, several cells thick.
- III. **Central cylinder** :
 - a. **Pericycle** an outer ring of sclerenchymatous cells, cells with thick walls.
 - b. **Vascular bundles** . . in an irregular ring, about 8.
 - (1) **Outer phloem**
Sieve-tubes, many, large.
Companion cells, small.
Bast parenchymatous cells, small.
 - (2) **Cambium**, easily perceived, walls stained blue.
 - (3) **Xylem**
Pitted ducts, very large.
Xylem parenchyma, etc.
 - (4) **Inner phloem**, like the outer phloem but less well developed. Not present in most Dicotyledons.
 - c. **Pith**, a central core of parenchyma.
 - d. **Medullary rays**, very broad, running from the pith radially outwards between the vascular bundles.

The above summary has been given for reference and as an aid in finding the phloem. There is not time to study all the tissues in detail. Your attention should be confined chiefly to the phloem and its sieve-plates.

In the **outer phloem** (next to the cortex) of one of the large central vascular bundles, with the **low-power** objective first, notice the fairly numerous, large, rather rounded, white-looking **sieve-tubes**. By adjusting the light properly with the iris diaphragm and by focussing up and down, you will be able to find **sieve-plates** in some of the sieve-tubes, although not in all. Notice how numerous the **perforations** in each sieve-plate are. At the side of a sieve-tube you may be able to distinguish a much smaller, somewhat flattened **companion cell**. Study some of the sieve-plates with the high-power objective.

Draw two sieve-tubes and their companion cells (if you have distinguished them), and in one sieve-tube show a **sieve-plate** with its **perforations**. The diameter of each sieve-tube should be about one inch.

D. THE STRUCTURE OF A FOLIAGE LEAF

(1) **Skeleton Leaves**.—The hardest parts of a leaf and the most resistant to decay are the vascular bundles, since in them many of the cells have walls which are both thick and lignified. Under natural conditions, it often happens that in a fallen leaf the epidermis, the palisade layer, and the spongy mesophyll are decomposed by bacteria before the vascular bundles have become appreciably rotten, so that the leaf becomes **naturally skeletonised**. The writer collected a number of such skeletonised leaves of a Poplar in a wood in England, and one of these leaves is provided for examination.

Examine the skeletonised leaf provided. Notice its **mid-rib**, its **lateral veins**, and its **very fine veins** which are united to form a close network. These veins are all **vascular bundles** along which water and other substances are conducted. If in a living leaf one or more vascular bundles should be severed, owing to the netted arrangement of the veins conduction of water is but little interfered with.

Make a drawing of a skeletonised leaf. Put in the **mid-rib**, the large **lateral veins**, and, in a small portion of the leaf, all the little ultimate **veinlets**.

(2) **Transverse Section through a Holly Leaf**.—Examine the stained and permanently mounted transverse section of the Holly leaf provided. A Holly leaf is very **leathery**, and this, as we shall see, is largely due to the great thickness of the **cuticle**. The following is a summary of the tissues present in the leaf.

Summary of Tissues in a Holly Leaf.

1. **Upper epidermis** one layer of cells thick, with a very thick unstained cuticle.
2. **Mesophyll** sometimes called the **chlorenchyma**, all the cells in the middle of the leaf between the upper epidermis and the lower epidermis except the vascular bundles. Its parts are:

of the mesophyll are the **palisade cells** and the **spongy parenchyma**, for it is the chloroplasts in these cells which manufacture sugar from carbon dioxide and water. The leaf as a whole must be considered as an organ specially adapted by its structure to do the work of photosynthesis.

Make a drawing of part of the transverse section of a Holly leaf, and show the chief tissues. The drawing should be about three inches in height and perhaps as wide as the page. Put in first the upper and lower epidermis. Then fill up the space between with the palisade cells, spongy parenchyma, vascular bundles, etc.

(3) **Transverse Section of a Leaf of *Tradescantia virginiana*** (Wandering Jew, Spiderwort).—This is provided to supplement the last slide. It is the leaf of a monocotyledonous plant. In it the palisade cells and the spongy parenchyma are not typically developed but are rounded cells and their chloroplasts have been destroyed in making the section. But the section shows two points very clearly: the **stomata** and the small **vascular bundles**.

In the lower epidermis look for stomata. In a single stoma, observe the two **guard-cells** and the **stomatic cleft** between them. In the leaf under each stoma notice the large **substomatic inter-cellular space**. This is in direct communication with the other intercellular spaces throughout the leaf tissue. It is *via* the stomatic clefts that gaseous interchange between the outer atmosphere and the leaf takes place.

Since in a monocotyledonous leaf the veins are parallel to one another, in a transverse section of such a leaf the **vascular bundles** must be **cut across transversely**. Notice that this is so in the leaf of *Tradescantia*.

Draw a stoma with its two guard-cells and stomatic cleft only if you have time to do so.

CHAPTER XXI

SPIROGYRA. THE FERN AND THE ALTERNATION OF ITS GENERATIONS

Materials and reagents supplied:

- (1) Some living *Spirogyra* filaments, if available; or
- (2) Some dead *Spirogyra* filaments which have been preserved in Bouin's solution;
- (3) A permanently mounted preparation showing *Spirogyra* in conjugation;

- (4) Iodine, dissolved in an alcoholic solution of potassium iodide, for staining blue-black any starch grains surrounding the pyrenoid bodies of Spirogyra ;
- (5) Some Fern spores ;
- (6) The prothallus of a Fern bearing antheridia and arche-gonia ;
- (7) A very young Fern Plant attached to a prothallus (to be passed around) ;
- (8) Part of a Fern leaf bearing sporangia.

A. SPIROGYRA

General Remarks.—Spirogyra, like Protococcus and Closterium, is **one of the green Algae**, but it differs from Protococcus and Closterium in having a **filamentous** (thread-like) **form** and in being **multicellular** instead of unicellular. Some Algae, e.g. the Red, Brown, and Green Sea-weeds, are **marine**, i.e. only grow in salt water, whereas Spirogyra and its allies only grow in fresh water. Hence, we can say that Spirogyra is a **fresh-water alga**.

Spirogyra is commonly known as **Green Silk** (from its slippery feel). In the summer it may be seen growing in bright-green slimy masses, often several feet in diameter, in the quiet low-lying water of ponds, ditches, springs, or slowly flowing streams. It is frequently associated with other green filamentous Algae. In bright sunny weather, when photosynthesis is active, **oxygen** is set free from the filaments in large quantities and forms **oxygen bubbles** which get caught between the filaments and buoy them up, thus producing a **scum** at the surface of the water. Sometimes the Algae which form a scum like that just described are called **Pond Scums**. Owing to its delicately foamy appearance, the scum formed by masses of Spirogyra filaments is known to many people as **Frog-spittle**.

In the genus Spirogyra there are about seventy species and a number of them have been found in Manitoba. The species are distinguished from one another by the width of their filaments, by the number and form of the chloroplasts in each cell, etc.

Examination of a Spirogyra Plant.—Mount two or three plants in a drop of water. If necessary, separate the filaments somewhat with the help of two needles. Then add a cover-glass.

With the naked eye, look at the preparation against a white background, and notice that a single Spirogyra plant has the appearance of a much elongated, unbranched, fine, green hair. Evidently a Spirogyra plant has a very simple form.

With the low-power objective of the microscope observe : (1) that a *Spirogyra* plant consists of a **long cylindrical unbranched filament** (thread) of very uniform diameter ; (2) that the filament is **septate**, the **septa** or cross-walls dividing it up into a **single row of elongated cells** ; (3) that **its two ends are alike**, so that the filament has no base or apex ; (4) that **its cells are all alike** in form and general structure ; and (5) that every cell in a filament contains one or more **spiral chloroplasts**.

With the high-power objective of the microscope examine some of the **individual cells** of a filament. Notice that each cell on its exterior is bounded by a definite **cell-wall** and that at its ends it is separated from each of its two neighbours by a **septum** or cross-wall. The outer wall of a *Spirogyra* filament consists of two different layers : (1) an **inner layer of firm cellulose**, which is the part of the wall you can readily perceive ; and (2) an **outer layer of mucus** which, without special staining, is more or less invisible. It is this outer wall-layer which causes *Spirogyra* filaments to feel slimy or mucilaginous when held between the fingers. Examine the exterior of a cell. By focussing up and down and observing the changes in the appearance of the surface, convince yourself that each cell is **cylindrical** in form.

Now examine the **cell-contents** of a cell. They resemble those of a typical parenchymatous cell. Just inside the cell-wall and pressed against it by the cell-sap is a thin **layer of cytoplasm** which we know as the **primordial utricle** ; while, occupying the centre of the cell and enclosed by the primordial utricle, is a **large vacuole** filled with **cell-sap**. This vacuole appears as a clear space and it can be made out by focussing downward from the top of a cell to its middle and then to its base.

Embedded in the primordial utricle are one, two, or more **chloroplasts**, the number varying with the species. Each chloroplast is a green ribbon, concavo-convex in cross-section, which winds round and round the cell in a spiral manner. Focus up and down and endeavour to **trace the winding of a chloroplast** around a cell. The two lateral edges of a chloroplast are straight in some species but jagged or serrated in others. What are the edges like in the species with which you have been supplied ? In each chloroplast there are a number of **pyrenoid bodies** which resemble those already observed in *Closterium*. A **pyrenoid** (from the Greek, *pyren* = a kernel, and *eidos* = resembling) **body** is a small, globular, colourless part of a chloroplast which appears to

be especially active in forming starch from sugar, for each pyrenoid body is surrounded by a thick white compact **sheath of starch grains**. Look for this sheath. Later on we shall study its reaction to iodine.

There is a single **nucleus** in each cell. It is always situated in the **centre of the cell**, and the **cytoplasm** in which it is embedded is connected with the cytoplasm of the primordial utricle by numerous delicate **cytoplasmic bridles**. These bridles often arise in the primordial utricle where the pyrenoid bodies bulge out the latter and, on their way to the nucleus which they suspend in the middle of the cell, they pass through the great central vacuole. In some species of Spirogyra the nucleus and the bridles can be readily perceived, while in others they are more or less hidden by the spiral chloroplasts. **Look for the nucleus** in your preparation.

Make a drawing of half a dozen Spirogyra plants very slightly magnified. Make the plants 3-4 inches long and represent each one by a single curvilinear line.

Make a second drawing of a single Spirogyra filament on a larger scale. Let the filament stretch right across the page and be not more than a quarter of an inch thick. Show the **outer wall** of the filament, the **septa**, and the **spiral chloroplasts**. The other details are not required.

Make a third drawing of just two adjacent cells of a filament but on a much larger scale. Each cell should be about **one inch in diameter**. Represent the **outer wall** and the **septa** by means of two parallel lines each. (a) **In one of the cells** show the **spiral chloroplast** or chloroplasts winding around the cell in the substance of the primordial utricle and indicate the **pyrenoid bodies** with their **starch sheaths**. (b) **In the other cell** represent semi-diagrammatically what can be seen in a median optical transverse section, namely, the **primordial utricle** with the **chloroplasts** seen in transverse section embedded in it, the **nucleus** surrounded by cytoplasm suspended in the middle of the cell by **bridles** of cytoplasm running between it and the primordial utricle, and the **vacuole** with its **cell-sap**.

Is Starch present in Spirogyra ?—Remove your preparation from the stage of the microscope and run a drop of **iodine** under the cover-glass. If starch is present in the filaments, it will show as a blue-black **sheath of starch** around each pyrenoid body. Each sheath is made up of many starch grains pressed closely together, but the grains cannot be seen as individuals.

Cell-division in Spirogyra.—Every cell of a Spirogyra filament can elongate and then divide into two daughter cells, thus increasing the length of the filament and the number of its cells. In this way a filament which begins its existence as a single cell may attain a length of several inches and come to consist of many hundreds of cells. If you were to **count the number of cells in one of your plants**, you might find that it exceeded 50. Cell-division in Spirogyra, preceded in the usual way by nuclear division, takes place **at night**, and the starch formed during the day is turned into sugar and used up in the process. The spiral chloroplasts grow in length as the cell containing them grows in length, and they divide when the cell divides.

Vegetative Multiplication.—In most species of Spirogyra there is no definite process for dividing up a filament into a number of separate daughter filaments; but, if a filament happens to be broken into two pieces by the attack of some aquatic animal or by some other accident, each piece grows and functions as a separate plant. In a few species only, the filaments sometimes break up spontaneously into their component cells and then each cell may develop into a new plant.

Reproduction by Conjugation.—Reproduction is effected in Spirogyra by a sexual process known as **conjugation**. In this process two filaments become yoked together and their cells unite in pairs. The products of the unions are known as **zygospores**. The essential features of conjugation will be set forth in the following summary of the reproductive processes in plants in general.

Summary of the Reproductive Processes in Plants.

Two main methods of reproduction are exhibited by plants: the **asexual** (non-sexual) and the **sexual**.

I. Asexual reproduction. In this a part of the parent organism is separated off and grows directly into a new plant. It may be accomplished vegetatively or by means of spores.

(a) **Vegetative reproduction.** The part separated off may be a more or less specialised portion of the vegetative region, e.g. a Potato tuber, a Hyacinth bulb, a new plant produced on the runner of a parent Strawberry plant.

- (b) **Asexual reproduction by means of spores.** In this process highly specialised single reproductive cells known as **spores** are produced, e.g. the spores produced in the sporangia of the Bread Mould, the conidiospores of a Blue-green Mould, the basidiospores of a Mushroom, the spores produced in the sporangia on the back of a Fern leaf, etc.

II. Sexual reproduction. This is brought about by special sexual cells known as **gametes**. A **gamete** (Greek *gamos* = marriage) is a unisexual protoplasmic body which is incapable of developing further and giving rise to a new individual until it has united with another gamete and has thus taken part in the conjoint production of a single cell known as the **zygote**. Sexual reproduction may be further subdivided according to the amount of differentiation of the gametes which fuse together.

- (a) **Conjugation.** In this process the **gametes** which unite are **similar in size and shape**, e.g. in Spirogyra and the Bread Mould. Conjugation is regarded as more primitive (a less highly evolved sexual process) than fertilisation. The zygote produced as a result of conjugation is often called a **zygospore**.

- (b) **Fertilisation.** In this process the gametes are clearly differentiated into **male** and **female**. The **male gamete** in many Algae (in Fucus but not in Spirogyra), in Mosses, Liverworts, Ferns, Horsetails, Club-mosses and certain Gymnosperms (Cycads and the Maiden-hair tree of China and Japan) is a small, ciliated, actively motile, naked cell known as a **spermatozoid** (corresponding to the **spermatozoon** of animals), while the **female gamete** is a relatively large, non-motile, passive cell known as an **ovum** or **egg-cell** (in Mosses and Ferns, etc., often also called an **oosphere**). The spermatozoid swims to the egg-cell, unites with it and thus fertilises it. The male and female nuclei contribute an equal number of chromosomes to the fusion nucleus. The **zygote** (in Mosses and Ferns, etc., also called the **oospore**), resulting from the union of a male and a female

gamete, develops into a new individual. In most Gymnosperms (Pines, Spruces, Balsam Fir, Tamarack, etc.) and in all Angiosperms (the monocotyledonous and dicotyledonous Flowering Plants) a **male nucleus** is conveyed to the egg-cell in an ovule by means of a special structure known as a **pollen-tube**.

Examination of a Preparation showing Spirogyra Filaments in Conjugation.—Examine the preparation supplied. Look all over it with the low-power objective and find the best place in it for observing some conjugated filaments. The dark oval bodies surrounded by a thick cell-wall are the **zygospores**, i.e. the products of the conjugation process. It will be seen that two conjugated filaments are connected with one another by **conjugation tubes**. Between two such filaments a certain amount of dirt is present. This was removed with the filaments from the pond where the alga was growing.

Conjugation in Spirogyra takes place in the following manner. Two filaments happen to be in contact side by side. They look alike and, at this stage, one cannot say that one is male and the other female. The cells of these neighbouring filaments then conjugate in pairs. Each of two cells which are in contact with one another sends out a blunt projection or **conjugation process**. The two conjugation processes meet at their tips, press against one another, and fuse, the wall at the point of contact becoming dissolved. In this way the two conjugating cells become joined together by a **conjugation tube** in which there is an open **conjugation canal**. Since a conjugation tube is formed by every pair of conjugating cells, a ladder-like appearance is given to the two filaments involved. Hence the conjugation process in Spirogyra is often called **scalariform conjugation** (Latin *scalaris*, pertaining to a ladder). As soon as the septum which at first lies across the middle of the conjugation tube becomes dissolved away, the two **protoplasts** (masses of protoplasm) of the conjugating gametes (sexual cells) come into contact and **become attached to one another**. Then one of the gametes begins to contract in size by the reduction of its great central vacuole to a smaller volume. This reduction, as recently discovered by Professor F. E. Lloyd of McGill University, is due to the action of small contractile vacuoles which are formed in the primordial utricle and rhythmically pump out the cell-sap from the great central vacuole. The

gamete which contracts first, thus taking the lead in activity, may be called the **male gamete**. When the male gamete has become much reduced in size, the protoplast of the other gamete, the **female gamete**, begins to contract (also by means of contractile vacuoles in the primordial utricle); and, as it contracts, it pulls the male gamete, which is attached to it, through the conjugation tube into its own cell. In the cell of the female gamete the two gametes undergo complete fusion, the cytoplasm fusing first and then the two nuclei. With the fusion of the two nuclei the conjugation process can be said to be complete. The product is an oval body called a **zygospore**. The zygospore surrounds itself with a **thick cell-wall** which has two outer layers and a delicate inner layer. At first the zygospore is green, but it soon becomes **dark brown**. It contains **oil drops** which serve as a reserve food material in preparation for germination after a period of rest. The zygospores of two conjugating filaments are all formed in **one** of the filaments. The filament which gave rise to the male gametes which passed through the conjugation tubes may be called the **male filament**, while the filament which received the male gametes and in which the zygospores have been formed may be called the **female filament**. At the end of the conjugation process, the male filament is empty and the female filament full of zygospores.

Owing to inequalities in the length and arrangement of the cells of two conjugating filaments, it often happens that, here and there, a cell has put out a conjugation process, but has **failed to obtain a partner**. **Look for some unmated cells** of this kind.

When the zygospores have been formed, the scalariform filaments sink to the bottom of the pool, the walls of the filaments rot away through the action of bacteria, and the zygospores are left scattered about on the mud. There they rest through the winter until the next spring and then they germinate. When a zygospore germinates, its two outer cell-walls break open and then the inner wall containing the protoplasm pushes outwards as a germ-tube. This germ-tube consists of a single cell, but it elongates and forms a rudimentary Spirogyra filament. The filament increases in length, undergoes cell-division and, in a few weeks, may come to consist of hundreds of cells. When a Spirogyra plant is very young, its two ends are **dissimilar**, there being an **apex** away from, and a **base** toward, the remains of the spore ;

but, with further development, carried out by means of **intercalary growth**, this distinction is soon lost, and thus the two ends of a well-grown Spirogyra filament come to be alike.

Make a drawing to illustrate conjugation in Spirogyra. Indicate the **male filament**, the **female filament**, the **conjugation tubes**, the **zygospores**, and an **unmated male or female gamete**. The drawing should be made the full width of the page, and each of the two conjugating filaments should be about one-third of an inch in diameter. Indicate what your drawing is meant to represent and the name of each part.

B. THE FERN

The Life-history of a Fern.—In the life-history of a Fern there are two very different kinds of plants to be considered, namely, the ordinary **Fern-plant** with which everyone is familiar and the **prothallus** (or prothallium), a tiny inconspicuous plant known only to botanists. The Fern-plant and the prothallus must be regarded as two **stages** or **generations** in a single life-cycle.

The life-cycle of a Fern may be briefly summarised as follows. A fertilised egg or **oospore** develops into a **Fern-plant** which has a root, a stem, and large leaves. On the backs of the leaves are produced clusters of **sporangia** and each **sporangium** contains 64 **spores**. The sporangia burst and liberate the spores, and the spores are scattered by the wind. With the production of these spores, the Fern-plant half of the life-cycle comes to a close. A **spore** germinates and produces a tiny, flat, heart-shaped, green plant only a few millimetres in diameter, in which neither root nor stem nor leaf can be distinguished. This is the **prothallus**. On its under side it has rhizoids which look like root hairs and serve for attachment and for absorption of water. Down the middle of the prothallus on its under side are produced two kinds of sexual organs: **antheridia** (male organs) and **archegonia** (female organs). Each **antheridium** produces 12–64 **spermatozooids** (male gametes) and each **archegonium** contains a single **oosphere**, or egg-cell. The archegonium has two chief parts: a basal **venter** in which the oosphere is contained and a projecting **neck** which at first is completely closed. On suddenly becoming immersed in a drop of rain-water or dew, the ripe antheridia and archegonia burst open. The spermatozooids are liberated from each antheridium and, with the aid of their cilia, they swim about in the water. The oosphere or egg-cell in each archegonium

remains in the venter of the archegonium, but access to it from the water is now possible owing to the fact that the archegonial neck has opened at its end and, through the loss of its central row of cells, has been converted into an open canal. A spermatozoid, in response to a chemical stimulus given by a malate (salt of malic acid) which diffuses out of the mouth of the archegonial neck, is attracted to an archegonium, swims down the **neck-canal**, comes into contact with the oosphere, and unites with the oosphere, thus fertilising it. The fertilised oosphere is known as an **oospore**. With the production of an oospore, the second half or prothallus half of the life-cycle of a Fern comes to a close. The oospore, on germinating, develops into a Fern-plant and thus is commenced a new life-cycle which will repeat what has just been described.

Alternation of Generations.—From the above account it will be seen that a **Fern-plant** (by means of a spore) **gives rise to a prothallus** and that a **prothallus** (by means of a fertilised egg or oospore) **gives rise to a Fern-plant**, and that this continues indefinitely, the sequence being: Fern-plant, prothallus; Fern-plant, prothallus; Fern-plant, prothallus; etc. Thus, in its life-cycle, a Fern exhibits the phenomenon known as an **alternation of generations**. This phenomenon occurs not only in Ferns but also in Mosses, Liverworts, Horsetails, Club-mosses, Coniferae, Monocotyledons, and Dicotyledons, although in the Seed-plants it is involved in the development of the ovule and seed and is much less apparent.

Sporophyte and Gametophyte.—The **Fern-plant generation**, as we have seen, ends in the production of **spores**. It may therefore be called the **sporophytic generation**, and the Fern-plant may be referred to as the **sporophyte**. The Fern-plant never bears any sexual organs and is therefore **asexual**, and we may therefore refer to the Fern-plant generation as the **asexual generation**.

The **prothallus generation**, as we have seen, ends in the production of a **fertilised egg-cell** or **oospore** which originates from the fusion of **two gametes**—a **male gamete** (the spermatozoid) and a **female gamete** (the egg-cell or oosphere). Hence the prothallus generation may be called the **gametophytic generation** and the prothallus may be referred to as the **gametophyte**. The prothallus bears sexual organs which contain sexual cells or gametes, and it is therefore a **sexual plant**; and we may therefore refer to the prothallus generation as the **sexual generation**.

We shall now endeavour to study a few phases of the life-cycle of a Fern.

(1) **Examination of Fern Spores.**—Examine the brown powder provided. It consists of spores which were liberated by the sporangia when a Fern leaf was allowed to dry on a sheet of paper. Mount some of the spores in water under a cover-glass and observe them with the microscope.

Notice the irregular or somewhat three-sided shape of the spores and their brown colour. **Make a drawing** of a few spores.

(2) **Examination of a Prothallus.**—Some spores, like those just examined, were sown on some soil (peat mixed with a little sand and soil does well for this purpose) in a small damp-chamber in the laboratory. They germinated readily and each one produced a prothallus. The prothallus supplied to you is several months old.

Place the prothallus with its under, rhizoid-bearing surface uppermost in a drop of water on a slide, and add a cover-glass. Examine with the low-power objective.

Observe that the prothallus is a more or less **flattened heart-shaped plant** and only one layer of cells thick except down its centre where it is several layers of cells thick. This central thickened part of the prothallus is known as the **cushion**. The cushion bears a number of long, brown, unicellular root-hair-like structures known as **rhizoids** and also the sexual organs. Examine the rhizoids carefully. Their function is to attach the prothallus to its substratum and to act as organs of absorption.

Observe that at the apex of the prothallus there is a bay or **sinus**. Just below the sinus is the **growing-point**, made up of a number of small cells which undergo division to produce new cells, thus providing for the growth in size of the prothallus.

Notice that the cells on the two sides of the prothallus are large and parenchymatous, with no intercellular spaces between them. In each cell there are many **chloroplasts** which resemble those already seen in a Moss leaf and in Flowering Plants. If photosynthesis has been actively carried on, you may be able to observe **starch grains** in the chloroplasts. Look for them.

At the margin of the prothallus are scattered here and there some little projecting **hairs**. These produce mucilage at their swollen apices.

Look for the sexual organs. The **antheridia** (male sexual organs) are produced first and some of them may be found in the

region of the rhizoids. An antheridium is a rounded projecting structure with a **wall** made up of a few wall-cells and an **internal cavity** filled with 12-64 **spermatozoids** (antherozoids). By focussing carefully the spermatozoids can be seen in the antheridium which contains them. If an antheridium is ripe and in good condition it will swell up in the water used as a mounting fluid and burst, and its spermatozoids will emerge. If you are fortunate, you may see some of the spermatozoids swimming about in the water. Each **spermatozoid** is coiled like a cork-screw, possesses a number of **cilia**, and moves rapidly through the water in a **spiral course**. A number of burst, empty, and discoloured antheridia may also be present in your preparation in the region of the rhizoids.

The **archegonia** (female sexual organs) are produced later than the antheridia, only when the prothallus is well grown, and just beneath the apical sinus. A single archegonium has two parts : a basal **venter** embedded in the cushion, and containing the oosphere, and a short **neck** projecting outwards from the cushion, downwards, and backwards. If the neck is still closed, it has a blunt rounded appearance at its end ; whereas, if it has opened, it will be funnel-shaped. Possibly your prothallus is still too young to have any archegonia, but you should look for these organs and study them if you find them.

Make a drawing showing a prothallus and some of its cells. Indicate the **cushion**, the **rhizoids**, the position of the **growing-point**, the **hairs**, the **antheridia**, and the **archegonia** (if you have seen them). Make the drawing the width of the page.

(3) **Examination of Sporangia on the Back of a Fern Leaf.**—Examine the leaf provided. With the naked eye notice the **sori** scattered about on the under side of the leaf, and then examine them under the microscope. The piece of leaf may simply be placed on a slide without water or cover-glass. A **sorus** may then be removed with a knife and examined in any way that seems best to you. A **sorus** is a **collection of small stalked sporangia** which are covered over and protected by a kidney-shaped scale called an **indusium**. The sporangia and the indusium which covers them are both developed on a **placenta** or little cushion of tissue situated immediately under a vein.

Remove some of the sporangia with a needle. Put them on a slide and observe them under the microscope, first when dry and then when mounted in a drop of water. Each sporangium

consists of a **stalk** and a **capsule**. The capsule is bi-convex. Its wall consists of a single layer of cells which are small and thin-walled except round the edge of the capsule, where they are large and their walls thickened and cutinised. This specialised layer of cells, which stands out prominently, has the function of contracting and bursting the sporangium open. It is called the **annulus**. On one side of the capsule it is incomplete. On this side are some broad cells which are separated when pulled upon by the annulus so as to form a mouth or **stomium** through which the spores can escape from the capsule. The maximum number of spores in a capsule is 64. Look for the spores. Perhaps in your material many of the capsules will have burst open and be empty.

Make drawings showing : (1) some **sori** on the back of a leaf, and (2) a **sporangium** (or two) with its **stalk**, **capsule**, **annulus**, **stomium**, and **spores**. Use your own judgment as to the scale of the drawings.

CHAPTER XXII

THE STRUCTURE AND SIGNIFICANCE OF FLOWERS, AND THE FLOWERS OF CERTAIN MONOCOTYLEDONS

Materials and culture supplied :

- (1) A Tulip flower and, if available, some older Tulip ovaries ;
- (2) A Hyacinth flower ;
- (3) A Lily-of-the-Valley flower ;
- (4) A Paper-white Narcissus flower ;
- (5) A Daffodil flower ;
- (6) A hanging-drop culture containing pollen-grains of the Easter Lily (*Lilium longiflorum*) or of a Paper-white Narcissus (*Narcissus Tazetta* var. *papyraceus*), to show germination and the structure of pollen-tubes ;
- (7) A strip of cardboard through which six holes have been punched in a line, for making a floral diagram.

The actual practical work begins (excepting a small part of p. 203) with the Section called *The Distinguishing Characters of Monocotyledons* (p. 207). The preliminary matter (pp. 199–207) should be read over carefully before the class begins.

I. REPRODUCTION IN FLOWERING PLANTS

Flowering Plants have received their English name from the fact that they produce special shoots concerned with reproduction, called **flowers**. Internationally, they are known to botanists as **Phanerogamia**. This name was given to them by the great Swedish botanist Linnaeus because their male and female organs (stamens and gynaecium) can be readily seen and recognised (Gk. *phaneros* = manifest, *gamos* = marriage), whereas in the **Cryptogamia** (Gk. *cryptos* = hidden, *gamos* = marriage) the sexual organs, although supposed to exist, had never been actually observed. Flowering Plants all produce complex reproductive bodies called **seeds** and are therefore often referred to as the **Spermaphyta** (Gk. *sperma* = a seed, *phyton* = a plant) or Seed-plants, in contradistinction to the Flowerless Plants, which are also called **Sporophyta** or Spore-producing plants.

Flowering Plants include not only the **Monocotyledons** and **Dicotyledons**, but also the cone-bearing plants or **Coniferae**. The **cones** of Pine-trees, etc., botanically speaking, are **unisexual flowers**. The male cones consist only of stamens which produce pollen grains, while the female cones consist of scaly carpels which bear ovules that develop into seeds. In the flowers of the Coniferae there are neither sepals nor petals present.

Flowering Plants may be classified as follows :

PHANEROGAMIA

(Flowering Plants, Spermaphyta.)

- I. **GYMNOSPERMAE**, ovules naked, not enclosed in an ovary.
 1. Coniferae, e.g. Pine, Spruce, Balsam Fir.
- II. **ANGIOSPERMAE**, ovules covered, enclosed in an ovary.
 1. Monocotyledones, e.g. Lily, Wheat, Palms, etc.
 2. Dicotyledones, e.g. Rose, Geranium, Bass-wood, etc.

The Inflorescence.

A mature Flowering Plant may be divided into two parts : (1) a **vegetative part** consisting of roots, stems which bear foliage leaves, and foliage leaves ; and (2) a **reproductive part** consisting of stems bearing flowers, and flowers, the function of the flowers being the production of complex reproductive bodies known as

seeds. The reproductive or floral part of a Flowering Plant is known technically as the **inflorescence**.

The **inflorescence** may consist of a single flower, as in the Tulip, or of many flowers massed together, as in the Hyacinth and Lily-of-the-Valley.

Summary of the Parts of a Mature Flowering Plant.

I. The vegetative part, non-sexual, devoid of flowers :

- | | |
|--------------------|--------------------------|
| (1) Root, | } ordinary green shoots. |
| (2) Stem | |
| (3) Foliage leaves | |

II. The reproductive part or inflorescence, sexual, bearing flowers.

- (1) A single terminal flower, e.g. the Tulip, or
- (2) Many flowers massed together, e.g. the Hyacinth.

The Flower.

A **flower** may be defined as a leafy shoot, of highly specialised structure, which performs the function of producing complex reproductive bodies known as **seeds**.

In the past, when men knew less than they do now and were more egocentric, it was thought that flowers had been created solely for the benefit and amusement of mankind. We know now that this view cannot be maintained ; for the geological record of the rocks teaches us that Flowering Plants appeared on the earth many millions of years before man, and that numerous species of flowering plants which once flourished over wide land areas have long been extinct, only their fossil remains affording evidence of their existence. In order to understand the true significance of flowers, we must lay aside any prejudices that we may have and study them in the same way as we have studied stems and leaves, i.e. **from the point of view of the plants which bear them** ; we must regard them as organs which are specially adapted by the structure and the functions of all their parts to secure the production of seed.

The **production of seed by a flower** is a very complex process. Certain tiny bodies called **ovules** are produced inside the **ovary**, a closed case forming part of the gynaecium or female part of the flower. In each ovule is an **egg-cell** or **oosphere** (ovum). Before an ovule can begin to develop into a seed, its egg-cell

must be fertilised by a **male nucleus** which is conveyed to it in a **pollen-tube**, the pollen-tube being produced by a **pollen-grain** which has germinated on the stigma of the gynaecium. As soon as the egg-cell in an ovule has been fertilised or, in other words, after the male and female gametes have fused together, the ovule grows rapidly in size and develops into a **seed**. When many of the ovules in an ovary have had their egg-cells fertilised, the ovary-wall is stimulated to grow in size and to develop into a **fruit-coat** or **pericarp**. A **fruit**, in its strict sense, is simply a **pericarp** (altered ovary-wall) and the seeds which it contains. The main events in the development of a typical flower may be thus summarised :

Summary of the Stages in the Development of a Flower.

1. **The flower develops** all its parts. It is protected by the calyx.
2. **The flower opens**, thus exposing the petals (which attract insects), the stamens (male organs), and the gynaecium (female organ).
3. **The anthers** of the stamens **dehisce**, i.e. open, so as to liberate the pollen-grains from the pollen-sacs.
4. **The stigma or stigmas become outspread** and thus ready to receive pollen grains (No. 3 may take place before No. 4, or No. 4 before No. 3, or Nos. 3 and 4 may take place simultaneously).
5. **Pollination takes place**, i.e. by some agent, e.g. the wind or insects, pollen-grains are removed from one or more anthers and are deposited on the stigma or stigmas. If the pollen is taken from the stamens to the gynaecium of the same flower, the flower is said to be **self-pollinated**, and we speak of **self-pollination** ; whereas, if the pollen is taken from the stamens of one flower to the gynaecium of another flower, the flower is said to be **cross-pollinated** and we speak of **cross-pollination**.
6. **The pollen-grains germinate** upon the stigma and each one produces a **pollen-tube**. The pollen-tubes penetrate into the tissues of the stigma and, by means of apical growth, grow downward in the style until they reach the ovary. They now penetrate into the cavity of the ovary and grow toward the mouths or **micropyles** of the ovules which they find unerringly, owing to

the fact that they are guided in their direction of growth by **chemical secretions** produced on the inner wall of the ovary and on the ovules themselves. The phenomenon is known as **chemotropism**. Each pollen-tube, near its elongating apical end, contains **two male nuclei** which were present in the pollen-grain at the time of its germination.

7. **Fertilisation of the egg-cells in the ovules.** On reaching the mouth or micropyle of an ovule, a pollen-tube forces its way into it and soon **comes into contact with the egg-cell**. Then the wall at the end of the pollen-tube becomes dissolved and disappears and the two male nuclei now pass out of the tube. **One male nucleus** approaches the female nucleus of the egg-cell and fuses with it, thus fertilising it and converting it into an **oospore**. The **other male nucleus** passes farther down the ovule and fuses with two other nuclei (**the secondary nuclei**), thus forming a single **endosperm nucleus**.
8. The **oospore** or fertilised egg-cell, which is a unicellular structure, now **undergoes cell-division** and develops into a multicellular **embryo**, i.e. a rudimentary plant consisting of a radicle, a plumule, and one or two cotyledons.
9. Whilst the embryo is developing, the **endosperm nucleus** with the cytoplasm surrounding it **undergoes division** to form the **endosperm**, a tissue which often more or less envelops the embryo.
10. **The whole ovule enlarges** greatly in size. Its integuments develop into a **testa**, and finally the **ovule becomes a seed**. The young seed consists of an embryo, an endosperm, and a testa.
11. **The seed ripens.** The seed enlarges to full size, and receives large amounts of soluble **food materials** which are stored up in solid form (starch, oil, cellulose, aleurone grains) either in the enlarged cotyledons of the embryo (Pea, Bean) or in the endosperm outside the embryo (Castor Oil, Coffee seed, Wheat grain). In seeds like the Pea where (when development is complete) there is no endosperm, the original endosperm which was present in the young seed becomes destroyed and replaced by the enlarging cotyledons of the embryo.

12. **The ovary wall enlarges** as the seeds are ripening and becomes a **pericarp** or **fruit-coat**. The pericarp together with the ripened seeds forms a **fruit**.
13. **Dispersal of the seeds.** When the fruit is ripe, either the pericarp **dehisces**, i.e. splits open, thus allowing the seeds to escape, or the pericarp remains **closed**, and the fruit, along with the seed or seeds which it contains, is carried away from the plant. When the seeds or the fruits containing seeds leave their place of development, the work of the flower has come to an end; it has produced and set free a set of bodies which can germinate and reproduce the species.

The Parts of an Inflorescence.

The main stem of an inflorescence is called the **peduncle**. The peduncle may be **unbranched** and bear a **single terminal flower** as in the Tulip or Prairie Crocus, or it may be **branched** and bear **many flowers** as in the Hyacinth and Lily-of-the-Valley. The ultimate branches of the peduncle to which the flowers are attached are known as **pedicels**. The branches of a peduncle including the pedicels arise, as a rule, in the axils of small leaves known as **bracts**. The one or two very tiny leaf-like structures which may be present on a pedicel are known as **bracteoles**.

Examination of Some Inflorescences.—Examine the inflorescences provided and make out their parts.

(1) **Tulip.** Notice that the inflorescence (the reproductive part of the plant) is very simple in that it consists of an unbranched peduncle and a solitary terminal **flower**. Each plant produces only one flower, but the flower makes up for its solitariness by its large size and brilliant colour. In *Anemone patens*, the Prairie Crocus, which has a blue flower, the inflorescence resembles that of the Tulip.

(2) **Hyacinth and Lily-of-the-Valley.** Notice that the peduncle or central axis of the inflorescence is a long, green, stem-like structure which is naked below and bears **white bracts** above, and that in the **axil** of each bract there is a **pedicel** which bends outwards and downwards and terminates in a drooping bell-like **flower**. This kind of inflorescence is known as a **raceme**.

The Parts of a Typical Flower.

The **pedicel** (stalk) of a flower looks like a stem and is morphologically equivalent to a stem bearing foliage leaves. Its apex

is the **receptacle** (also called the **thalamus** or **torus**) of the flower, and on this receptacle are set 4 **whorls** (circles) of **floral leaves**: (1) the **calyx**, (2) the **corolla**, (3) the **androecium**, and (4) the **gynaeceum**.

(1) **The Calyx**.—The outermost set or whorl of floral leaves is known as the **calyx** (Gk. *kalyx* = a cup) which is made up of separate parts called **sepals**. It is usually green. During the development of a flower, i.e. in the flower-bud stage, it envelops and protects all the other parts of the flower. Its function is therefore **protective**. The sepals are the most leaf-like parts of the flower.

(2) **The Corolla**.—The second set or whorl of floral leaves attached to the receptacle is known as the **corolla** (Lat. = a little crown) which is made up of separate parts called **petals**. The petals are large, highly coloured, often scented, leaf-like structures which make the flower conspicuous and serve to attract insects. In some flowers (Columbine, Larkspur, Violet, etc.) the corolla has one or more of its petals hollowed out to form a spur-like **nectary** in which is secreted a sweet juice called **nectar** much sought after by bees, butterflies, moths, and other insects. Bees suck up the nectar, pass it into their crops and there, by means of an enzyme, convert it into **honey** which they then regurgitate and store in the waxen cells of their hives. The corolla functions by attracting insect agents of pollination and by assisting these agents by its peculiar form in bringing about the transfer of pollen from the stamens to the stigma. Insect-pollinated flowers are known as **entomophilous flowers**, while wind-pollinated flowers are known as **anemophilous flowers**. In the latter the petals are always small, inconspicuous, odourless, and devoid of nectaries.

(3) **The Androecium**.—The third set or whorl of floral leaves is known as the **androecium** (Gk. *aner*, *andros* = a man) which is made up of separate parts called **stamens**. This is the **male floral whorl**, and the stamens may be considered as the **male organs** of the flower. A stamen has two parts: a stalk or **filament** and an **anther**. The anther usually has two distinct lobes and each lobe may be somewhat divided longitudinally by a slight groove. In each anther there are **four** longitudinally elongated **pollen-sacs** and each pollen-sac contains some hundreds or thousands of **pollen-grains**. There are two pollen-sacs in each of the two anther lobes. When an anther is ripe,

each lobe **splits down one side** along the median groove and the two flaps of tissue on each side of a split bend backwards and thus **expose the pollen** contained in two of the pollen-sacs. The pollen is made up of **tiny pollen-grains**. In most flowers this pollen dust is **yellow** in colour, e.g. in the Buttercup and the Dandelion where it is **vivid yellow**, but in the Hawthorn it is **snow-white**, in the Mignonette **crimson**, in the Clover **russet-brown**, and in the Poppy **jet-black**. The androecium functions by providing the pollen-grains which are necessary to bring about fertilisation of the ovules in the gynaecium.

(4) **The Gynaecium**.—The fourth and innermost set or whorl of floral leaves is known as the **gynaecium** (Gk. *gynaikeion* = the woman's house) or **pistil** (L. *pistillum* = a pestle), and it is made up of separate parts known as the **carpels**. This is the **female floral whorl** or **female part** of the flower. Each **carpel** is considered to be a **much modified leaf**. In primitive flowers, e.g. the Buttercup and the Prairie Crocus, the carpels are all **separate** from each other and in each carpel the two edges of the leaf have become fused together to form a hollow chamber below known as the **ovary**, a little medium stalk known as the **style**, and a terminal part called the **stigma**. In the ovary there is always one or more (usually many) tiny whitish oval bodies known as **ovules**. These are **potential seeds**, but they cannot develop into seeds until their egg-cells (one in each ovule) have been fertilised by a male nucleus.

In flowers more advanced than the Buttercup and Prairie Crocus, e.g. the Tulip, Hyacinth, Narcissus, and Daffodil, the **carpels**, instead of being separate from one another (the **apocarpous** condition), are all **fused together laterally** (the **syncarpous** condition), so that in the more highly evolved flowers the gynaecium consists of a **single compound structure**. Thus, in the Tulip, Hyacinth, Narcissus, and Daffodil, there are **three** carpels fused together laterally. The three ovaries have become fused to form **one large ovary with three chambers** in it, each chamber containing many ovules; the three styles have become fused to form a **single style**, while the three stigmas still show as **three lobes to the stigma** which terminates the style.

The **ovary chamber** in a syncarpous gynaecium may be **single**, or it may be divided up into separate parts known as **loculi** or **cells** (the term cell is here used in the same sense as in a prison). Thus in the tricarpeal gynaecium of the Violet the ovary is

unilocular or **one-celled**, whereas in the tricarpeal gynaecium of the *Narcissus* and its allies the ovary is **trilocular** or **three-celled**.

The **ovules** are developed at certain, often swollen, portions of the ovary wall known as **placentae** (sing. *placenta*). In the *Violet* the **placentation** is said to be **parietal** (on the outer wall), whereas in the *Narcissus* it is said to be **axillary**, i.e. on the central axillary column of the ovary.

The function of the **ovary** is to produce ovules, to protect the ovules as soon as they come into existence and whilst they are developing into seeds, and to provide a means by which the seeds may be liberated from the flower, thus permitting of their dispersal.

The function of the **stigma** is to receive pollen-grains and provide for their germination. It is usually a sticky or feathery structure, and there are **often just as many stigmas as there are carpels** or there are **loculi** in the ovary.

The function of the **style** is to place the stigma in the best position in the flower for the reception of pollen brought by the wind or insects, and to connect the stigma with the ovary. Pollen-tubes grow down the interior tissues of the style on their way to the ovules.

The following is a summary of the parts of a typical flower as just discussed :

Summary of the Parts of a Typical Flower.

- I. The **pedicel** (flower-stalk), a stem-like structure terminating in a **receptacle** (**thalamus**, **torus**), which bears the floral leaves.
- II. The **whorls of floral leaves** (organs) and their parts
 - (1) The **calyx**, made up of **sepals**, often green, protective.
 - (2) The **corolla**, made up of **petals**, coloured, attractive.
 - (3) The **androecium**, made up of **stamens**, male organs.
 - (4) The **gynaecium**, made up of **carpels**, the female organ or **pistil**.

Stamen	{	Filament (stalk). Anther , two-lobed with four pollen-sacs containing pollen- grains .
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Gynaeceum.....	{	Stigma, receives pollen-grains.
		Style, a stalk connecting the stigma with the ovary.
		Ovary, a chamber, single or divided, containing one or more ovules.

The Perianth.

In some flowers, e.g. Tulip, Hyacinth, Lily-of-the-Valley, Narcissus, and Daffodil, the sepals instead of being green are coloured like the petals or are **petaloid** and, as in all these flowers except the Tulip, the sepals and petals may be united together laterally to form a **tube**. In such flowers as these it is convenient to speak of the **calyx and corolla** together as the **perianth**. Thus, in a Tulip, we can say that the perianth consists of two whorls, an outer whorl of petaloid sepals and an inner whorl of petals ; while, in the Hyacinth, Narcissus, etc., we can say that the perianth consists of a **perianth-tube** with six **terminal segments**, the three outer ones representing the calyx and the three inner ones the corolla.

Perianth.....	{	Calyx
	{	Corolla

II. THE DISTINGUISHING CHARACTERS OF MONOCOTYLEDONS

Monocotyledons are distinguished from Dicotyledons : (1) by the embryo having **one cotyledon** instead of two ; (2) by the **vascular bundles** in the stem being **numerous** and **scattered** instead of few and arranged in a ring ; (3) by the **absence of any cambium** between the xylem and phloem of the vascular bundles and, consequently, by the absence of any secondary thickening of the stem ; (4) by the leaves having **parallel** instead of netted **veins** ; and (5) by the number of parts in each whorl of floral leaves in the flower being typically **three** instead of five or four.

In the Tulip provided notice : (1) that the leaves have **parallel veins**, and (2) that in a thin cross-section of the end of a stem (made with a sharp pen-knife and looked at with the naked eye) the **vascular bundles** are **numerous** and **scattered**. By these two characters alone we can be quite sure that the Tulip is a Monocotyledon, and we can predict that further observation would

show that the embryo of the Tulip has but one cotyledon, that the vascular bundles have no cambium, and that the stem never develops a ring of secondary xylem or any secondary phloem.

Do not pull any of the flowers to pieces until this is required by the instructions.

The Flowers of some Monocotyledons.

To-day we shall study the flowers of some typical Monocotyledons, namely, the Tulip, the Hyacinth, the Lily-of-the-Valley, the Paper-white Narcissus, and the Daffodil. All these plants are bulbous and their flowers, already formed, lie hidden in the bulb until the spring, when they rapidly unfold at the expense of the food materials stored up in the fleshy scale-leaves.

The Tulip.

The Tulip, *Tulipa Gesneriana*, was introduced into Europe from Turkey about 1554. Clusius, the famous Dutch herbalist, introduced the Tulip into Holland, where he grew tulip plants for sale and sold them at high prices. The Dutch went wild over Tulips, and there was a famous Tulip-mania in Holland from 1634 to about 1638. People speculated in tulip bulbs just as they do now in stocks, and as much as 13,000 florins was paid for a bulb of the variety *Semper augustus*. Finally, the Dutch government stepped in and passed anti-Tulip-speculation laws.

With the help of the summary of the parts of a flower already provided and with the aid now to be given, **make out all the floral whorls** of the flower and **how each whorl is arranged** in respect to its neighbours.

Notice that the **perianth** consists of six segments, three outer ones and three inner ones, the three inner ones **alternating** in position with the three outer ones.

The **three outer segments** of the perianth evidently make up the **calyx** and are the **sepals**. Since the sepals instead of being green are brightly coloured, we must describe them as **petaloid**. The sepals in the Tulip undoubtedly assist the petals in making the flower attractive to insects.

The **three inner segments** of the perianth evidently make up the **corolla** and are the **petals**. They were enclosed by the three sepals when the flower was in the bud stage of its development.

Notice that the **androecium** is made up of six **stamens**, and that each stamen consists of a **filament** and an **anther**. If the anthers

are still intact, each one will be seen to be bilobed ; but, if they have **dehisced** (opened), it will be seen that each one is covered on both sides by yellow **pollen-grains**. In the mass these grains are often referred to simply as **pollen** or as **pollen-dust**.

The **six stamens** are not in one whorl as in our typical flower but in **two whorls**. There is an outer whorl of **three slightly older stamens** which alternate in position with the three petals, and an inner whorl of **three slightly younger stamens** which alternate in position with the three outer stamens. The stamens of the inner whorl are set just a very little bit **higher on the receptacle** than the stamens of the outer whorl.

So far, we have considered a whorl of sepals, a whorl of petals, and two whorls of stamens. We now come to the fifth and last whorl of floral leaves, namely, the **gynaeceum**. This consists of an elongated, three-sided, greenish or yellowish **ovary** and **three stigmas**. Notice that there is **no style** present, a somewhat exceptional condition. It appears that here the ovary is long enough to put the stigmas in the right position for receiving pollen, a style therefore not being required.

Notice that the three stigmas **alternate** in position with the three inner stamens. As there are **three stigmas**, this suggests that the gynaeceum is **tricarpellary**, which is the fact, and that possibly there are three loculi or cells in the ovary. Later on, when the ovary is examined in cross-section, we shall see that the ovary is in fact **trilocular** (3-celled).

Make a Floral Diagram of a Tulip Flower.—A floral diagram is a **ground plan of a flower** which shows the **number and arrangement of all the floral leaves**. These leaves are to be shown on a frame-work of circles and lines.

The Tulip flower has five whorls of floral leaves and, therefore, for its floral diagram, **five circles** are required. These could be made most easily with a pair of compasses ; but in the absence of this instrument, the five circles may be constructed as follows :

Take a strip of cardboard, as provided, through which six holes have been punched in a line. Insert a needle through one of the end holes and thus pin the strip to a page in your drawing-book. Then revolve the strip five times with a pencil-point inserted through a different hole at each successive revolution.

When the five circles have been made, draw **three diameters** across them, one from **back to front** and the other two so that the three lines form six angles at the centre, **each angle being 60°**.

When this has been done, it will be seen that the area included by the outermost circle has been divided up into six equal sectors.

We now have five circles and three diametric lines on which to represent all the floral leaves of the flower. Now **look at the flower again** and represent each of its five floral whorls on the floral diagram.

Put in the **calyx** first. Represent the **sepals** in diagrammatic cross-section. Let **one sepal** be shown in section **at the top of** the frame-work along the outermost circle. The **two other sepals** will then necessarily be placed right and left in the bottom half of the circle. To distinguish the sepals from the petals, each sepal may be represented diagrammatically as having a **mid-rib**.

Now put in the **corolla**. The three **petals**, shown in cross-section, must be placed on the next circle to that just used, and they must be made to **alternate** with the sepals. **No mid-ribs** should be shown.

We now come to the **androecium**. On the third circle put in the **outer whorl of three stamens**. These must be shown **alternating** with the petals. Each stamen should be represented by a **two-lobed anther** seen in cross-section, the two lobes facing toward the centre of the circle.

Next, put in the **inner whorl of three stamens** alternating with the three stamens of the outer whorl.

Finally, we come to the **gynaeceum**. For this draw a **triangle** to represent the **outline of the ovary wall**. One point of the triangle will be at the **top** of the innermost circle. The further representation of the ovary must be left until after the drawings of individual parts of the flower have been made.

Make drawings, natural size, of the following parts of the Tulip flower : (1) a single **sepal** (remove one) ; (2) a single **petal** (remove one) ; (3) a single **outer stamen** (remove one) ; (4) a single **inner stamen** (remove one) ; and (5) the **gynaeceum** on the top of the **receptacle** which crowns the **peduncle**. Indicate the name of each floral leaf drawn and the parts of the stamen (**filament**, **anther**) and gynaeceum (**ovary**, **stigmas**).

Examination of Pollen-grains.—With a needle **remove some pollen-grains from an anther**, place the dust on a slide, add a drop of water and a cover-glass, and then examine the preparation with a microscope.

Each pollen-grain has two walls, an outer and an inner, and its cell-lumen is filled with dense cytoplasm in which there are two nuclei (the **tube nucleus** and the **generative nucleus**), but these cannot now be distinguished. Pollen-grains, in origin, are morphologically equivalent to the spores of Ferns. A stamen may be thought of as a very much reduced fern-leaf having only four sporangia (the four pollen-sacs).

Germination of Pollen-grains.—Pollen-grains, when placed on a stigma, germinate within 1–2 hours; and they also germinate just as readily in suitable solutions of sugar. This may be observed in the **hanging-drop preparation** which is provided.

The **hanging-drop preparation** provided consists of a van-Tieghem cell with a hanging drop of a 10 per cent. **cane-sugar solution** in which some pollen-grains were placed about half an hour before the practical class began its work. The pollen-grains were taken from a stamen of the large white **Easter Lily** (*Lilium longiflorum*) or from a stamen of the **Paper-white Narcissus** (*Narcissus Tazetta* var. *papyraceus*). Often a tear-like drop can be seen on the very large stigma of the Easter Lily, and a fraction of such a drop of stigmatic fluid is an excellent medium in which to germinate Easter Lily pollen-grains. Some hanging-drop cultures of this kind have been prepared, but not enough to provide one for every member of the class.

Examine the hanging-drop culture. Perhaps some of the pollen-grains therein have already germinated; but, if not, you should be able to observe germination before the end of the practical period.

In a germinated pollen-grain one sees the **grain** still covered by an outer sculptured wall but with its smooth inner wall growing outwards to form a rapidly elongating **pollen-tube**. The pollen-tube is filled with **cytoplasm** which is clear at the apex of the tube but densely granular elsewhere. Very often the cytoplasm exhibits **protoplasmic streaming** in a very striking manner. Look for this movement with the high-power objective.

There are **three nuclei** at the end of each pollen-tube but, unfortunately, they cannot be distinguished without staining: a **tube-nucleus** at the growing-point and **two male nuclei** a little way behind the growing-point. These two male nuclei came into existence by the division of the generative nucleus which, along with the tube-nucleus, was present in the ungerminated pollen-grain.

Make a drawing of some germinated pollen-grains. Indicate the **pollen-grain**, the **pollen-tube**, the **apical growing-point**, and the **cytoplasm**.

Dissection of the Gynaeceum.—With a pen-knife or scalpel cut the **ovary** across the middle transversely. Notice, with the naked eye or with a pocket lens, that the ovary has **three loculi** or **cells**, and that in each loculus there are **two ovules** visible. The ovules are attached to the central column of the ovary. We may say, therefore, that the **placentation of the ovules is axillary**.

To see the structure of the ovary just described more clearly, take a razor or sharp scalpel and make a **thin transverse section of the ovary**. Put the section on a slide (mounting fluid and cover-glass unnecessary) and look at it with the **low-power objective** of the microscope. The three loculi of the ovary and the ovules in each loculus attached to the axillary part of the ovary should now be clearly perceived.

Completion of the Innermost Whorl of the Floral Diagram.—Now complete your floral diagram. Show the **wall of the ovary**, the **three loculi** (or **cells**), and **two ovules** in each loculus attached to the ovary wall along the axillary column.

Dissect the ovary further, and observe that there are **two rows of closely packed ovules in each loculus**.

Remove some ovules from the ovary, put them on a slide, and observe them with the **low-power** objective of the microscope. Notice their shape. Each ovule contains an **egg-cell** (oosphere) but this is hidden within it near its mouth or micropyle and cannot be seen. Only after fertilisation of the egg-cell can an ovule develop into a seed. We may therefore describe an ovule as a **potential seed**.

The Hyacinth.

The Hyacinth, *Hyacinthus orientalis*, which has come to us from the Mediterranean region, is closely related to the Tulip and belongs to the **Liliaceae** or Lily family.

The inflorescence of the Hyacinth, as we have seen, consists of a peduncle bearing bracts in the axils of which there are pedicels bearing flowers. This kind of inflorescence is known as a **raceme** and it is evidently much more complex than that of a Tulip, which consists simply of a single terminal flower.

Make a sketch of the raceme of the Hyacinth and show the pendulous flowers hanging from their pedicels.

Study of a Single Flower of the Hyacinth.—With the floral diagram of the Tulip before you for comparison, make out the structure of a single Hyacinth flower. Would the floral diagram of the Tulip do for the floral diagram of the Hyacinth ?

Notice that the flower of the Hyacinth is **pendulous** instead of being upright as in the Tulip.

Notice that the perianth agrees with that of the Tulip in that it consists of **six** segments, **three outer ones** representing the **sepals** of the **calyx** and **three inner ones** representing the **petals** of the **corolla**.

Notice that the **perianth** differs from that of the Tulip in that the sepals and petals are not free from one another but are united laterally more than half way up so as to form a **perianth-tube**.

Pull the perianth-tube so that it splits downwards to its base into two halves. Find the **stamens** and **count their number**. Normally, six stamens are present in each flower but, under cultivation, **abnormalities** are frequent and occasionally, therefore, the number of stamens exceeds six.

Notice that the stamens are **inserted on the perianth-tube** just at the top of its basal swollen part (in the Tulip, the stamens were inserted on the receptacle). Each stamen consists of an elongated **anther** attached to a very short **filament**. Make sure that the filament is really present.

In the development of the Hyacinth flower, the three stamens alternating with the corolla lobes develop a little sooner than the other three stamens. Hence we conclude that the six stamens of a Hyacinth flower resemble those of the Tulip, i.e. they consist of an **outer whorl** of three stamens alternating with the petals and an **inner whorl** of three stamens alternating with the outer whorl of stamens.

Notice that the **gynaecium** consists of a somewhat **trifid stigma**, a **style** which looks like three styles laterally united, and a swollen **ovary**. A cross-section of the ovary reveals that it is **trilocular**, that its **loculi** contain many **ovules**, and that the **placentation** of the ovules is **axillary**. Evidently the gynaecium, as in the Tulip, is **tricarpeous**.

From the above study it should have become clear that the flower of the Hyacinth and that of the Tulip, although to a casual observer so very unlike, are in reality built up on exactly the same general plan. This plan may be represented by : (1) the **floral diagram** already drawn, or (2) by the following **floral**

formula which is self-explanatory : Ca.3, Co.3, A.3+3, G.3. The bar below the last 3 indicates that the ovary is **superior**, i.e. that the perianth segments are inserted on the receptacle below the gynaeceum.

The Liliaceae.

The Tulip, Hyacinth, Easter Lily, Prairie Lily, Tiger Lily, Day Lily, Solomon's Seal, Wild Smilax, Onion, and Asparagus all belong to the great natural family known as the **Liliaceae** which includes some 2,000 species.

The **floral formula** of the Liliaceae is that already given for the Tulip and Hyacinth, namely : Ca.3, Co.3, A.3+3, G.3 ; and the **floral diagram** for the Liliaceae is like that already constructed for the Tulip.

The Paper-White Narcissus.

A flower of the Paper-white Narcissus, *Narcissus Tazetta* var. *papyraceus* (one of the so-called Polyanthus Narcissus varieties), is provided for examination. Make out its structure. Notice :

(1) The **ovary**, which is green and swollen, can be seen below the perianth-tube without any dissection of the flower, i.e. the ovary is **inferior** in position, instead of superior as in the Tulip and the Hyacinth. This is a **fundamental difference**.

(2) The **perianth** arises at the top of the ovary and consists of a long, white, rather narrowly-cylindrical perianth-tube which, at its top, divides into six segments turned outwards at right angles. Here, again, we have **three outer lobes** or **sepals** representing the calyx and **three inner lobes** or **petals** representing the **corolla**.

(3) Around the mouth of the perianth-tube and projecting from it is a collar-like structure, known as the **corona**. This is not present in a Hyacinth flower. The **corona** is a special out-growth of the perianth-tube which is characteristic of all species of the genus *Narcissus* and is not present in related genera.

(4) The **stamens**, as in the flowers already studied, are six in number and arranged in **two whorls**. There are **three upper** (outer and older) stamens which can be seen projecting at the mouth of the perianth-tube (within the corona), and **three lower** (inner and younger) **stamens** a little way down the perianth-tube. **Split the perianth-tube to its base** and find the three inner stamens. As in the Hyacinth, the six stamens are inserted on the perianth-tube.

(5) The **gynaeceum** consists of an inferior dark-green, swollen ovary, a long thin **style**, and **three** small **stigmas** or stigmatic lobes. Cut the **ovary** across transversely and observe that, as in the flowers previously studied, it is **trilocular**, that each **loculus** contains **many ovules**, and that the **placentation** is **axillary**.

From the above observations it will have been gathered that the floral diagram for a Narcissus flower would be exactly like that for a Tulip or a Hyacinth.

The Daffodil.

The flowers of the Daffodil, *Narcissus Pseudo-Narcissus*, which, as Shakespeare says, "comes before the Swallow dares, and takes the winds of March with beauty," are perhaps the most lovely of all those which appear in early spring. Botanically speaking, a Daffodil is nothing more or less than a highly specialised Narcissus. The Daffodil and the Paper-white Narcissus are but two species of the same genus.

Examine the Daffodil flower provided and notice that it resembles a Paper-white Narcissus flower in all essentials, the main difference being that, whereas in the **Narcissus** the **corona** is relatively **short** and inconspicuous, in the **Daffodil** the **corona** is produced forward into a **very large and splendid yellow trumpet**. The corona, indeed, is the most conspicuous part of the perianth.

Notice that in the Daffodil, as in the Narcissus, there is an **inferior ovary**, a **perianth-tube** which has six lobes (3 sepals and 3 petals) which are turned outwards at right angles, and that the **corona** is a special outgrowth of the relatively short perianth-tube. Notice further the six stamens (3 + 3) inserted on the perianth-tube. To make this observation **split the corona and perianth-tube to the base**.

Notice further that the **gynaeceum** consists of a large green **inferior ovary**, a long yellow cylindrical **style**, and **three stigmas** or stigmatic lobes. Cut the **ovary** across and notice that its structure is just like that of the Narcissus, the Tulip, and the Hyacinth, i.e. it is **trilocular**, with **many ovules** in each loculus, the **placentation** of the ovules being **axillary**.

The Amaryllidaceae.

The Narcissus, the Daffodil, the Snowdrop, the Amaryllis, and the Agave all belong to the large natural family known as the **Amaryllidaceae**, which includes 700 species.

The **Liliaceae** and **Amaryllidaceae** are two closely related monocotyledonous families. In both families: there are five whorls of floral leaves; the perianth consists of 3 sepals and 3 petals; the stamens are 6 (3 + 3) and the gynaecium is tri-carpellary with a trilocular ovary. There is, however, a **very important difference** between the two families. It is this: that, whereas in the **Liliaceae** the ovary is **superior**, in the **Amaryllidaceae** the **ovary is inferior**. The Amaryllidaceae may be considered as more advanced in the evolution of their flowers than the Liliaceae or, in other words, the Liliaceae is the more primitive of the two families.

There is no difference between the **floral diagram** of the Liliaceae and of the Amaryllidaceae, but there is a difference in the **floral formulae** of the two families. Where the floral formula of the Liliaceae is:

$$\text{Ca.3, Co.3, A.3+3, G.}\bar{3},$$

the floral diagram for the Amaryllidaceae is:

$$\text{Ca.3, Co.3, A.3+3, G.}\bar{3},$$

the position of the bar of the last figure indicating that the ovary in the Liliaceae is **superior** and in the Amaryllidaceae **inferior**.

The Lily-of-the-Valley.

Some flowers of the Lily-of-the-Valley have been supplied.

In the light of what has just been said above, determine for yourself **to which of the two families**, the Liliaceae and the Amaryllidaceae, the Lily-of-the-Valley belongs. Record the reason or reasons for your decision in your book.

CHAPTER XXIII

THE FLOWERS OF SOME DICOTYLEDONS

Dicotyledonous flowers supplied:

- (1) Garden Stock, *Matthiola incana*, single flowers;
- (2) Garden Stock, double flowers;
- (3) Sweet Pea, together with a strip of cardboard punched with holes, for making a floral diagram;
- (4) Violet;

- (5) Marguerite, *Chrysanthemum frutescens*, also called the Paris Daisy.

If any of the above flowers should not be available, the following flowers may be used as substitutes :

- (6) Snapdragon ;
(7) Primula ;
(8) Cineraria.

The actual practical work begins with the Section called *The Garden Stock as a Type of the Cruciferae* (p. 220). The preliminary matter (pp. 217-220) should be read over carefully before the class begins.

I. THE FAMILIES OF FLOWERING PLANTS

In the last practical class we became acquainted with two natural families of Monocotyledons, namely, the **Liliaceae** and the **Amaryllidaceae** ; and to-day we shall study representatives of at least four of the following dicotyledonous families : the **Cruciferae**, the **Violaceae**, the **Leguminosae**, the **Scrophulariaceae**, the **Primulaceae**, and the **Compositae**. All these and some other important families of Flowering Plants (Phanerogamia) may be classified as follows :

Classification of Some of the More Important Families of Flowering Plants.

(a) GYMNOSPERMAE

Class Coniferae.

- (1) *Taxaceae* e.g. Yew.
(2) *Pinaceae* e.g. Pine, Spruce, Balsam Fir,
Tamarack (Larch).

(b) **ANGIOSPERMAE**

Class **Monocotyledones.**

- (1) *Palmae* e.g. Date Palm, Coconut Palm.
- (2) *Araceae* e.g. Calla Lily (Arum Lily), Monstera.
- (3) *Liliaceae* e.g. Tulip, Hyacinth, Easter Lily.
- (4) *Amaryllidaceae* ... e.g. Narcissus, Daffodil, Snowdrop.
- (5) *Gramineae* e.g. Wheat, Corn, all wild Grasses.
- (6) *Iridaceae* e.g. Iris, Blue-eyed Grass.
- (7) *Orchidaceae* e.g. Lady's Slipper Orchid.

Class Dicotyledones.I. **Polypetalae** or families with **separate** petals.

- (1) *Ranunculaceae* ... e.g. Buttercup, Prairie Crocus, Larkspur, Aconite, Hellebore, Columbine.
- (2) *Cruciferae* e.g. Stock, Wallflower, Sweet Alyssum, Shepherd's Purse, Cabbage, Radish.
- (3) *Violaceae* e.g. Violet, Pansy.
- (4) *Rosaceae* e.g. Rose, Hawthorn, Apple, Pear, Cherry, Plum, Peach, Strawberry, Raspberry.
- (5) *Leguminosae* e.g. Sweet Pea, Bean, Clover, Sweet Clover, Alfalfa, Caragana.

II. **Gamopetalae** or families with **united** petals.

- (1) *Solanaceae* e.g. Potato, Tomato, Tobacco, Petunia, Henbane, Datura, Deadly Nightshade.
- (2) *Scrophulariaceae* ... e.g. Snapdragon, Foxglove, Musk, Veronica, Pentstemon, Toad-flax, Calceolaria.
- (3) *Labiatae* e.g. Mint, Thyme, Sage, Lavender, Rosemary.
- (4) *Primulaceae*..... e.g. Primula, Primrose, Cowslip, Oxlip.
- (5) *Compositae* e.g. Sunflower, Marguerite, Daisy, Cineraria, Golden-rod, Aster, Dandelion.

II. THE DISTINGUISHING CHARACTERS OF DICOTYLEDONS

Dicotyledons are distinguished from Monocotyledons : (1) by the embryo having **two** cotyledons instead of one ; (2) by the **vascular bundles** in the stem being **few** and **arranged in a ring** instead of being numerous and scattered ; (3) by the **presence**, instead of the absence, of a **cambium** between the xylem and the phloem of the vascular bundles, and by the products of cambial activity, namely, **secondary xylem** (one or more annual rings of wood) and **secondary phloem** ; (4) by the leaves being **net-**

veined instead of parallel-veined ; and (5) by the number of parts in each whorl of floral leaves in the flower being typically **five** or **four** instead of three.

The Flowers of some Dicotyledons.

To-day we shall study the flowers of some typical Dicotyledons, namely, the **Garden Stock**, the **Sweet Pea**, the **Marguerite** and the **Violet**. If any of these flowers should not be available, the **Snapdragon**, the **Primula**, or the **Cineraria** will be used as a substitute.

In treating of the floral morphology of the flowers just named and of flowers in general it is convenient to make use of certain descriptive terms which will now be explained.

Explanation of Certain Terms.

A. In respect to the **position of the floral whorls on the receptacle**:

(1) A **hypogynous** flower (hypo = **below**) is one in which the **gynaeceum** (pistil) occupies the highest position on the receptacle, while the petals and sepals are inserted on the receptacle at successively lower intervals **below** the gynaeceum. In such a flower the **ovary** is said to be **superior** ; e.g. Buttercup, Prairie Crocus, Tulip, Hyacinth, Stock, Violet, Snapdragon, Primula.

(2) A **perigynous** flower (peri = **around**) is one in which the receptacle is hollowed out to form a saucer-like or cup-like structure. The gynaeceum is inserted in the middle of the cup, while the stamens, petals, and sepals are inserted **around** the gynaeceum on the rim of the saucer-like or cup-like extension of the receptacle. The **ovary** in such a flower is still said to be **superior** ; e.g. Cherry, Raspberry, Rose. The Sweet Pea is weakly perigynous.

(3) An **epigynous** flower (epi = **upon** or **above**) is one in which the receptacle is hollowed, as in the perigynous flowers, and the ovary of the gynaeceum is concealed in the concavity thus formed and is **fused with and coherent to the sides of the cavity**. It thus becomes impossible to separate the wall of the ovary from the receptacular cup, and the stamens, petals, and sepals appear as if they were inserted **upon the top of** or **above** the ovary. The ovary in such a flower is said to be **inferior** ; e.g. Narcissus, Daffodil, Marguerite, Cineraria.

B. In respect to the **union of parts** :

1. A **polysepalous** calyx is one in which the sepals are **separate** from one another, e.g. Buttercup, Tulip, Stock, Violet.

2. A **gamosepalous** calyx is one in which the sepals are **united** laterally, e.g. Sweet Pea, Primula.

3. A **polypetalous** corolla is one in which the petals are **separate** from one another, e.g. Tulip, Buttercup, Stock, Violet, Sweet Pea, Rose.

4. A **gamopetalous** corolla is one in which the petals are **united** laterally, e.g. Snapdragon, Primula, Marguerite, Cineraria.

C. In respect to **floral symmetry** :

1. An **actinomorphic** (= star-like) or **regular** flower is one which can be divided into two equal halves along **several planes**, e.g. Tulip, Hyacinth, Lily-of-the-Valley, Narcissus, Daffodil, Stock, Primula.

2. A **zygomorphic** (= two-sided) or **irregular** flower is one which is bilaterally symmetrical, i.e. one which can be divided into two equal halves along only **one plane of symmetry** (usually the antero-posterior plane), e.g. Violet, Pansy, Sweet Pea, Snapdragon, Lady's Slipper Orchid.

A. The Garden Stock as a Type of the Cruciferae.

The Garden Stock, *Matthiola incana*, will be studied as a type of the Cruciferae. Its seeds, under cultivation, give rise to plants with **double flowers** and plants with **single flowers** (like that provided). Only the single flowers yield seed, so that the double flowers all come from seed obtained from single flowers. A **double** Stock flower is an **abnormal flower** in which the number of petals is increased beyond four and in which stamens and pistil are absent. Every double flower may be called a **monstrosity**.

One of the most striking characters of the Cruciferae is that the petals are **cruciform**, i.e. are arranged to form a cross. Hence the name **Cruciferae** (= the cross-bearers).

Draw a flower of the Garden Stock in face view, so as to show the cruciate arrangement of the four petals.

The **inflorescence** of the Stock is a **raceme** resembling that of the Hyacinth and Lily-of-the-Valley, except for the fact that there are **no bracts** and the flowers on the ends of the pedicels are **erect** and not hanging down.

Now **study the Stock flower provided** and make out its different parts, beginning with the calyx.

Notice that the flower as a whole is **actinomorphic** (regular) and not **zygomorphic** (irregular). In this respect it is primitive. By its **colour** and **scent** the flower is made attractive to butterflies and certain other insects. Can you detect any scent?

Calyx.—Note that the calyx is made up of **four sepals**, $2 + 2$, and is **polysepalous** (sepals separate). The **two lateral sepals**, known by their **pouches** at the base, form an **inner whorl** of the calyx and are inserted higher on the receptacle than the other **two** unpouched **median sepals** which form an **outer whorl** of the calyx. (At first, it looks as though the median sepals are higher on the pedicel than the lateral, but this is not so.) The **pouches** at the base of the lateral sepals are little downward extensions of the sepals which act as **receptacles to hold the nectar secreted by the nectaries**. The four sepals are erect and somewhat coherent above so that they form a **tubular sheath** around the corolla.

Corolla.—Note that the corolla is made up of **four petals** arranged in a whorl alternating with the four sepals. The petals are **separate** from one another and the corolla is therefore **poly-petalous**.

Dissection of the flower.—With the fingers carefully pull away from the flower the **two outer sepals** (the non-saccate ones), then the **two inner sepals** (the saccate ones) and then the **four petals**. Notice the hollowed base of each saccate sepal and that each petal consists of a pink **blade** and a yellowish-green **claw**.

The **stamens** and **nectaries** have come into view and may now be conveniently examined.

Androecium.—This consists of six stamens, $2 + 4$, hypogynous, separate. There are **two outer short stamens** opposite the lateral sepals (the saccate ones), and there are **four inner long stamens**. Thus the androecium consists of two whorls. The short stamens are much shorter than the gynaecium and the long stamens considerably longer than the gynaecium. Notice that each stamen has an elongated yellow **anther** and a greenish **filament**.

The **nectaries** are little **green fleshy extensions of the receptacle**. There are **two** nectaries present, one around the base of the filament of each short stamen. The nectaries project into the saccate bases of the lateral sepals and **excrete nectar into the sacs**. Butterflies, etc., visit the flower to rifle the nectar which thus accumulates.

Gynaeceum.—This occupies the centre of the flower and is the highest floral whorl. With the fingers **pull away from the flower** the two short outer stamens and then the four long inner stamens ; and then the gynaeceum will be seen seated **on the top of the receptacle** which terminates the **pedicel**.

The gynaeceum consists of an almost cylindrical (oval in cross-section) whitish **ovary** and **two stigmas**. There is **no style**, as in the Tulip. In a young opened flower the two stigmas, which are somewhat triangular in shape, are pressed together ; but, as the flower becomes older, they diverge from one another.

The gynaeceum is **bicarpellary**, syncarpous, superior. The ovary has two parietal placentae bearing numerous ovules. The placentae are connected and bisected by a partition or **septum** which divides the ovary into two chambers, so that the ovary is **bilocular**. For reasons which will not be entered into here the septum has been termed a **false septum**.

To **make out the two chambers** in the ovary, the septum, and the ovules, a cross-section should be made with a sharp scalpel or razor. The section should be set dry on a slide and should be examined with the low-power objective.

Pollination and the Floral Mechanism.—Every flower should be conceived of as a **floral mechanism** constructed in such a way that pollination of its stigmas will be brought about by some agency, e.g. the wind, insects, water, etc. We shall now discuss the floral mechanism of the Garden Stock.

In the Garden Stock, the four sepals, somewhat coherent above, closely surround the four claws of the petals and form a tube about 15 mm. long with nectar at the base on two sides. In the wild species the corolla-tube may become half-filled with nectar. The Small Tortoise-shell Butterfly, *Vanessa urticae*, which has a tubular tongue or proboscis 14–15 mm. long, has been observed visiting the flowers, sucking up the nectar and passing from flower to flower, thus doubtless bringing about their **cross-pollination**. The **short stamens**, which do not reach up to the stigmas, **serve for cross-pollination**. An insect endeavouring to get at one of the secreting nectaries will be dusted with some of the pollen from the adjacent short stamen and will transfer it to the stigma of another flower of the same species. The **four long stamens**, which are longer than the gynaeceum, have their anthers placed immediately beneath the opening of the flower and their anthers dehisce introrsely. They serve for **self-pollination** which is

effected by the pollen falling on the stigma or by the visits of insects.

Floral formula of the flower of a Garden Stock.—From the study of the flower which has just been made, we may summarise the structure in the following formula :

$$\text{Ca.2} + 2, \text{Co.4}, \text{A.2} + 4, \text{G.2}.$$

The formula indicates that the flower has six whorls of floral leaves and is hypogynous. **Record this formula** in your book.

The Cruciferae.

The Cruciferae is a large family in which there are some 1200 species. In addition to the Garden Stock it contains such well-known flowering plants as : Wallflower, Honesty, Shepherd's Purse, French-weed, Cabbage, Cauliflower, Turnip, Radish, Mustard, and Cress.

The floral formula for the Cruciferae is the same as that given above for the Garden Stock. The **distinguishing characters** of the Cruciferae may be summarised as follows :

Cruciferae. Herbs. Leaves alternate, exstipulate. Inflorescence racemose, usually without bracts. Flower regular, hypogynous. Sepals $2 + 2$, polysepalous. Petals 4, polypetalous. Stamens $2 + 4$, two short and four long. Carpels 2, syncarpous ; ovary divided by a false septum connecting two parietal placentae ; ovules numerous. Fruit usually a siliqua. Seed, no endosperm.

Double Flowers of the Garden Stock.

Examine a **double flower** of the Garden Stock. Note the **numerous petals**. All double flowers, such as those of the Stock, the Rose, the Daffodil, the Begonia, etc., are from the botanical point of view **monstrosities**. They could not persist under natural conditions. Usually their stamens were reduced in number and their petals greatly increased in number. In the middle of a **double rose** there are usually some floral organs which are **half petal and half stamen**.

Has a double flower of a Garden Stock any stamens or pistil ?—Examine your flower carefully and try to find stamens and a pistil (gynaeceum). If these organs are absent, how does a double-flowered Garden Stock plant reproduce itself ?

B. The Sweet Pea as a Type of the Leguminosae.

The Sweet Pea, *Lathyrus odoratus*, will be studied as a type of the **Leguminosae**. Owing to its beauty and fragrance it is considered by flower-lovers as the queen of the large genus to which it belongs. It has been in general cultivation since about A.D. 1700. Originally, there were three colour varieties: a **purple** variety in Sicily, a **white** variety in Sardinia, and a **pink and white** (bicolor) variety in Ceylon. These have been intercrossed and now there are some hundreds of horticultural varieties.

The **Sweet Pea Plant** is a climbing annual with a winged stem and compound leaves. Each leaf has two lanceolate stipules on its petiole, while its lamina is divided into one pair of lateral leaflets, one pair of lateral tendrils (taking the place of leaflets) and one terminal tendril.

The **inflorescence** of the Sweet Pea is a 2-4-flowered raceme. The peduncle bears very much reduced (almost completely suppressed) **bracts**, in the axil of each of which is a curved **pedicel** bearing a large and showy **flower**.

Study the Sweet Pea Flower provided and make out all its different parts.

The Sweet Pea flowers obtained for this lesson were grown in Ontario and, as a result of packing, they may have become a little crushed out of their natural shape. If the **large upper petal** should have been pressed down on the other petals, flatten it with your fingers somewhat and **turn it backwards** into a **vertical position**.

Draw the flower from one side, natural size. Your drawing will show the green pedicel, the short (1-2 mm. long) green obconical **receptacle**, the gamosepalous green **calyx**, with three of its five segments, the large **upper petal** (the **standard**) with its posterior edge vertical, and **one of the lateral petals** (a wing).

The **flower as a whole**, when regarded in face view, is evidently **zygomorphic** or **irregular**, i.e. it can be divided into two equal halves along **one plane** only. Notice that this plane is antero-posterior in position. Later, after cutting vertically downwards into two halves, it will be possible to see that the flower is **weakly perigynous**. The showiness of the flower is due to the great development of the corolla and to the diversity of form and curious arrangement of the petals. When the flowers were growing in Ontario they were scented, but they are now

odourless. Smell them. The loss of fragrance took place as a result of subjection to a low temperature in the box in which they were shipped to Winnipeg. Violets lose their odour in a similar manner.

Calyx.—This is **gamosepalous**, the five sepals being united laterally to form a 5-toothed cup. Count the sepals and observe their union.

Corolla.—This is **polypetalous**, the petals being separate from one another. At first view, one might suppose there were only four petals (look at the flower in face view), but there are, as we shall see, **five petals**. Make them out as they are described.

The **posterior petal** is the largest of the five petals and is known as the **standard** (vexillum). In the wild varieties it has a vertical position and makes the flower very conspicuous to insects. The **two lateral petals** are called the **wings** (alae) and each is about half the size of the standard. The **two anterior petals** have separate claws (which cannot at present be seen) but they cohere along the lower edges of their blades so as to form a **boat-shaped structure** known as the **keel** (carina).

The manner in which the parts of the flower are folded up before expansion is known as **aestivation**. Notice that in the Sweet Pea the standard overlaps the two wings, and the wings overlap the two keel-petals. Overlapping is known as **imbrication**. The **aestivation** of the petals of the corolla of the Sweet Pea is therefore **descending-imbricate**.

The two wings and the keel of the corolla are **interlocked**. When a bee alights on the flower it uses the two wings as a platform. The weight of the insect **depresses not only the wings but, owing to interlocking, the keel also**. To observe this, hold the flower by its stalk in the left hand, and then do what a bee does: take hold of the two wings (with a finger and thumb of the right hand) and pull them downwards. You will then find that the keel becomes depressed along with the wings. Why is this so? The keel-petals each have a **hollowed groove** on the upper side of the inner half of the blade into which fits a **ridge** projecting from a wing, and it is owing to this interlocking arrangement that the weight of a bee depresses both wings and keel at one and the same time.

The **androecium**, with all its stamens, and the **gynaecium** are **hidden from view** in the chamber made by the two keel-petals; but you will have found that, on depressing the wings and keel,

the stamens and gynaecium **spring out of the chamber** and can then be readily seen.

Now begin to **dissect the flower**. Pull off the keel and lay it on your page. Notice that the keel has **two separate** basal stalks or **claws**, one belonging to each keel-petal. Notice further the **groove** on the outer and upper side of each of the keel-petals. Now divide the keel down its middle line so as to separate it completely into its two component petals. Keep the parts.

Now remove the two **wings**. Notice that each has a large **blade** and a short **claw**. Look at the **ridge** on the blade of each wing, which fitted into a hollow of a keel-petal. Keep the wings.

Now remove the **standard**. Notice that its claw is short and broad and that its blade is far larger than that of a wing-petal.

Draw : (1) the **standard**, (2) a **wing**, and (3) a **keel-petal**. Lay these petals flat on your page over the places where you want to draw them, mark their outlines with pencil-points or lines to serve as guides, then remove the petals and complete the drawings.

In the flower there are now left the tubular green calyx, the androecium, and the gynaecium. Pull the **calyx** so as to split it down to its base and then **pull it away** from the **receptacle**, so as to remove it completely. The receptacle will now be more apparent as an obconical green swelling at the top of the pedicel. We are now in a position to study the androecium.

Androecium.—The number of **stamens** is **ten**. Count them to make sure. Thus there are twice as many stamens as there are sepals or petals. Of these ten stamens the **posterior** (upper) one is **separate from the rest**. With a needle or knife **raise up the posterior stamen**, so that its **filament** can be seen all the way from the receptacle to the anther. The filament is about an inch long. The other nine stamens have filaments which are **free** from one another at their ends (for about one-third of an inch) but which are **united laterally** below their ends (for about two-thirds of an inch) so as to form a **staminal tube**. This tube is white and not complete, for it is open along the top by a slit which corresponds in position to the filament of the single free posterior stamen already studied. Notice that all the ten stamens are turned upwards at their ends.

The top of the flat receptacle enclosed between the staminal tube and the contracted stalk of the ovary is **glandular** and excretes **nectar** which accumulates in the space between the staminal tube and the ovary stalk. The receptacle, therefore,

in addition to bearing the whorls of floral leaves, acts as a **nectary**. The receptacle will be bisected and examined later on.

Depress the staminal tube, so that it becomes free from the ovary which it has enclosed.

Make a drawing showing the androecium from the side. The drawing should include the **pedicel**, the **receptacle**, the upper **free stamen**, and the nine **combined stamens**. The **gynaeceum** may also be put into the same drawing if you can see it clearly.

Gynaeceum.—This occupies the centre of the flower and consists of a long, green, laterally compressed, hairy **ovary**, a long upturned flattened and twisted **style**, and a very small terminal **stigma**. The upper half of the style, which may be called the **stylar brush**, is covered on one side with a series of stiff hairs which look obliquely upwards. The function of this stylar brush is to **sweep out the pollen from the keel** and to deposit it on the under side of a bee's body when the bee depresses the wing-petals and keel. At first, in the young flower, the style is straight, but as the flower develops the style becomes twisted, so that the stylar brush which at first faced the pedicel now comes to look toward one side of the flower. The **stigma** is a **tiny greenish-yellow plate**, not more than one-third mm. long, which projects from and crowns the top of the long style and receives pollen on its smooth and moist surface. Find the stylar brush and the stigma.

Look at the base of the ovary (after the staminal tube has been dissected away) and notice that the ovary arises from a short thin **stalk**. It is in the space around this stalk that the nectar secreted by the upper flat green surface of the receptacle accumulates, in readiness for the visits of bees.

The gynaeceum in the Sweet Pea and in all other Leguminosae is very simple, for it consists of **one single carpel**. The edges of the carpellary leaf, as it were, have been folded upwards, brought together, and fused together so that the carpel has formed a closed case which we call the **ovary**. The ovary thus comes to be **unilocular** (1-celled).

The **placenta** upon which the ovules are borne is made up of the fused edges of the carpellary leaf and is situated along the upper median line of the ovary. The ovules hang down into the ovary chamber from their placenta and there is a double row of them, one row corresponding to each of the fused edges of the carpellary leaf. This is best seen when the ovary is becoming

a fruit (a pod), for then the ovules are swelling into seeds and are more easily detected. Since the placenta is on the outer wall of the ovary, the **placentation** of the ovules is said to be **parietal**.

Draw the gynaecium from one side. Show the **ovary** with its hairs, the upturned **style** with its **stylar brush**, and the terminal **stigma**.

Now slit open the ovary from below and find the ovules hanging down in the ovary-chamber or **loculus**. When the ovary is very young, its two sides are so closely pressed together that the cavity has to be looked for carefully. A transverse section of an ovary, made with a razor and examined with the low-power objective of the microscope, would show the ovary-wall, the cavity between its two sides, and possibly an ovule.

The Fruit.—A fruit is a developed ovary together with its ripened seeds. In the Sweet Pea the fruit is known as a **legume** and popularly is called a **pod**. We studied the **pod of a Garden Pea** in our first practical class. **Turn to your drawing of it** and compare it with the ovary of the Sweet Pea which you have just investigated.

Pollination and the Floral Mechanism.—The peculiar structure of any flower as a whole and the significance of the form and position of each of its parts can not be fully understood until one becomes acquainted with the relations of the flower with insects, the wind, or some other external agent of pollination. Just as one asks how a mouse-trap works, how a gramophone produces music, or how an electric bell rings, so one should ask how a flower manages to bring about pollination, i.e. the transfer of the pollen-grains from the stamens to the stigmas, as a preliminary to seed-production. Until this question has been answered satisfactorily, one's knowledge of any flower is still very incomplete.

The flower of the Sweet Pea, like those of all its relatives, is specially adapted to pollination by means of **bees**. A bee, attracted by the peculiar colour, form, and scent of the corolla, alights on the flower in such a way as to use the two wing-petals (the alae) as a **landing-stage**. The weight of the bee depresses the wing-petals, which in turn drag down the keel (carina). This downward movement of the keel leads to the exposure of the upwardly directed stigma, the stylar brush, and of the anthers which inevitably come into contact with the under-surface of the bee's body. The bee thrusts its long tongue into the slit

on the upper (posterior) face of the staminal tube and sucks up the nectar which has been excreted by the receptacle and has accumulated between the lower part of the staminal tube and the stalk of the ovary. When the bee leaves the flower, the two wings and the keel rise up again and assume their original position, thus again hiding the stamens and stigma from view. A bee, visiting flower after flower, may thus effect **cross-pollination**. Sweet Pea flowers which do not happen to be visited by bees effect their own **self-pollination**. The pollen from the ten stamens comes into contact with the stigma of the same flower automatically.

The wings and keel protect the nectar and pollen from rain and from robber insects (unbidden guests) which would not effect pollination. The nectar is concealed so that it can only be reached from above (from the slit along the top of the staminal tube). We have seen that the wing-petals and keel can be depressed by the weight of a bee and that, when the bee flies away from the flower, the wing-petals and keel spring back to their old positions. What causes this return? This problem you may try to solve for yourself when further material is available.

The Flower of the Sweet Pea is Weakly Perigynous.—To make out this point, take another flower, if it is available, and cut it into two halves down its plane of symmetry. By this means the receptacle and the staminal tube will be bisected. Look at one of the flower-halves and observe that the **top of the receptacle** is **flat**, that the ovary is borne on its thin stalk in the middle of the receptacle, and that the staminal tube, petals, and calyx are situated **not under the gynaecium** but **around it**, a little distance from it, on a flattened extension of the receptacle. Hence we may say that the flower of the Sweet Pea is not hypogynous but is weakly **perigynous**.

Notice the **space** between the base of the staminal tube and the thin stalk of the ovary. It is in this space or cavity that the nectar excreted by the receptacle accumulates.

Floral Diagram.—Make a floral diagram of a Sweet Pea flower. Four concentric oval lines will be required, one for the calyx, one for the corolla, one for the androecium, and one for the gynaecium. Make a circle very lightly with your cover-glass dish and then convert the circle into an oval by extending it above and below. Now find the **lowest point** on the oval and

from this point **divide the oval up into five equal arcs**. Join the five points on the oval to the centre of the circle by means of lightly-drawn straight lines. Put in an inner circle with the help of a ten-cent piece, and convert it into an oval like the outer oval. Then divide the five lines between the two ovals into three equal parts and, with the help of the guide-points, add the other two ovals required.

Another method for making the four ovals is as follows. Use the **strip of cardboard with punched holes** that you employed for making a floral diagram of the Liliaceae and construct (1) an upper set of four semi-circles about one central point and then (2) a lower set of four semi-circles about another central point situated half an inch below the first. Then join the two sets of semi-circles so as to make four ovals.

Now, with the help of the frame-work already made as a guide, put the **calyx** into the floral diagram. Look at a flower again, if one is available, and notice that two of the sepals lie right and left at the back of the flower. Put in these two posterior sepals first and then the other three. Each sepal should be represented as having a mid-rib. Since the calyx is gamosepalous, the edges of the five sepals may be joined up to indicate this.

Now put in the **corolla**. Construct the large posterior petal, the **standard**, first. Then put in the two **keel-petals** next, showing how they meet. Finally put in the two **wing-petals** (alae). These should be shown with their upper ends enclosed by the standard and with their lower ends enclosing the keel. Thus the diagram comes to show not only the number and position of the petals but their mode of imbrication.

Now put in the **androecium**. The ten stamens should be represented on the third circle by means of their anthers. Each anther should be made bilobed with the lobes looking toward the middle of the flower, i.e. the anthers should be represented as **introrse**. All the **nine lower stamens** should be **joined by lines** to indicate that their filaments are united to form a staminal tube.

Finally, put in the **gynaeceum**. Represent this by a cross-section of its **ovary**. Show the two edges of the carpellary leaf meeting above and turning downwards, and show **one ovule** projecting downwards in the ovary chamber.

To show that the flower has arisen in the axil of a bract attached to the peduncle, one may put a **little circle** at the **top** of the floral

diagram to represent the **peduncle**, and a little **cross-section** of a **leaf** at the **bottom** of the floral diagram to represent the **bract**.

Record the floral formula for a Sweet Pea flower. This formula may be represented as follows :

$$\text{Ca.}(5), \text{Co.}5, \text{A.}(5+4)+1, \text{G.}\underline{1}.$$

The brackets enclosing the first figure 5 indicate that the calyx is gamosepalous, and the second set of brackets indicates that nine stamens are united. The number of stamens is indicated as $(5 + 4) + 1$ instead of $(9) + 1$ because a study of their **earliest development** on the receptacle shows that the ten stamens, when in the most rudimentary condition, consist of **two whorls**, an outer whorl of 5 stamens alternating with the petals and an inner whorl of 5 stamens alternating with 5 outer stamens. **Later** in development the stamens become fused laterally and form a staminal tube and then they all seem to arise at the same level on the receptacle, i.e. to form a **single whorl**. Hence, in the floral diagram, the ten stamens are inserted on a single oval. In the flower of some other Leguminosae the five outer stamens are longer than the five inner ones, but in the Sweet Pea this distinction is not apparent.

The Leguminosae.

The **Leguminosae**, to which the Sweet Pea belongs, is the second largest family of Flowering Plants, coming second only to the Compositae (Sunflower family). It contains about 7,000 species. The Leguminosae live in every kind of soil and climate and in every country, and they include herbs (Pea, Bean, Clover), shrubs, (Caragana, Gorse), trees (Acacia, Locust tree), climbers, water-plants, etc.

The **roots** of the Leguminosae exhibit swellings or **nodules** (tubercles) in which live vast numbers of nitrogen-fixing bacteria (*Bacterium radicola*). The **fruit** of the Leguminosae is typically a **legume** or pod which dehisces along what are called the dorsal and ventral sutures into two valves. The seed has no endosperm and the reserve food materials are stored up in the much swollen **fleshy cotyledons** of the embryo.

Economically, the Leguminosae is a most important family. It yields seeds for food (Pea, Bean, Vetch, Pea-nut), many fodder plants (Alfalfa, Sweet Clover, Clover), fibres, dyes (haematoxylin or logwood, indigo), gums (gum tragacanth), oil, and valuable timbers (Rosewood).

The Leguminosae are made up of three large sub-families : the **Mimosoideae** (no species in Canada), the **Caesalpinioideae** (no species in Canada), and the **Papilionatae**, and many authors raise these sub-families to family rank. Then the Papilionatae, which includes the Sweet Pea and all Pea-like plants, is called the **Papilionaceae** (*papilio* = a butterfly). The distinguishing characters of the Pea division of the Leguminosae may thus be summarised.

Leguminosae (Papilionaceae). Herbs, shrubs or trees. Leaves alternate, stipulate, and usually compound. Flowers poly-petalous, perigynous, papilionaceous. Stamens 10, one separate and the other 9 united, or all united. Gynaeceum monocarpellary. Fruit a legume. Floral formula :

$$\text{Ca.}(5), \text{Co.}5, \text{A.}(5+5), \text{G.}\underline{1}$$

or

$$\text{Ca.}(5), \text{Co.}5, \text{A.}(5+4)+1, \text{G.}\underline{1}.$$

C. The Marguerite or the Cineraria as a Type of the Compositae.

The **Compositae** is the largest and most successful family of Flowering Plants, and it includes over 10,000 species. Among these are the following : Sunflower, Canada Thistle, Sow Thistle, Golden-rod, Daisy, Dandelion, Lettuce, Chicory, Dahlia, Black-eyed Susan, Marguerite, and Cineraria. We shall take as our type of the Compositae either the Marguerite or the Cineraria, whichever happens to be available.

The **Marguerite**, *Chrysanthemum frutescens*, is also known as the Paris Daisy. It grows wild in the Canary Islands, but it is cultivated as a shrubby pot-plant in Europe and North America. The **Cineraria** comes from South Africa and our cultivated varieties have been produced by hybridisation.

One of the most characteristic points about the Compositae is that the **inflorescence** is a **capitulum** (L. = a little head), i.e. that the reproductive part of the plant consists of a large number of tiny flowers which are set closely together on the convex or flattened end of the peduncle and are protected by a set of **bracts** known collectively as the **involucre**.

Examine the inflorescence provided (the Marguerite is white and yellow, the Cineraria purple). By non-botanists the inflorescence is always referred to as a single "flower"; but, for

the botanist, the so-called "flower" of a Marguerite or Cineraria is made up of a **large number of tiny flowers** known as **florets**.

If not already done, divide the inflorescence down the middle into two equal halves. Notice that the **peduncle** of the inflorescence is expanded at the apex so as to form what is called a **disk**. In the Marguerite the disk is **convex** above, while in the Cineraria it is **flattened** above.

On the outside of the inflorescence attached to the disk observe the imbricating **bracts** (green in Cineraria, green with brown margins in Marguerite) which collectively form what is called the **involucre**. The involucre bracts cover and protect the florets when the capitulum is very young.

Notice that the **flowers** (florets) **on the disk are of two distinct kinds** : (1) **ray florets**, which form a single series at the periphery of the capitulum, known by their broad strap-shaped corollas (white in Marguerite, purple in Cineraria), and (2) **disk florets**, more numerous much less conspicuous flowers (**yellow** in Marguerite, **yellow** and **purple** in Cineraria), which occupy all the space on the disk within the circle of the ray florets.

(1) **A Ray Floret**.—Remove a ray floret from its attachment to the disk (dissect it away carefully, do not leave the basal ovary behind), and examine it in detail.

Notice that the ray floret has an **inferior ovary**. In the Marguerite this is greenish-white, somewhat compressed and winged, while in the Cineraria it is green and more cylindrical.

The **calyx** in a ray floret of Marguerite is an ill-developed ridge at the top of the ovary, the ridge being extended upwards into about three whitish teeth. In the Cineraria the calyx of a ray floret is a green ridge at the top of the ovary, i.e. it is almost non-existent.

The **corolla** in a ray floret arises at the top of the ovary. Its basal part is tubular and greenish, but distally it expands into a broad or strap-shaped structure or **ligula** which is white in the Marguerite and purple in the Cineraria. The corolla-tube (judging by comparison with other flowers) is made up of five petals; while the ligula (judging by the **three tiny terminal teeth** seen in the Marguerite) is made up of an extension of **three** of the petals. It is the ligulae of the ray florets which give to the capitulum as a whole its rayed appearance and which make it so conspicuous.

The **gynaecium** consists of an **inferior ovary** (already seen),

a **style**, and **two** terminal **diverging stigmas**. Find the end of the style protruding out of the corolla-tube and the two divergent stigmas. Slit the corolla-tube downwards to its base and observe that the style springs from the top of the inferior ovary. The presence of **two** stigmas indicates that the gynaecium is **bicarpellary**.

The **androecium** in the ray florets of the Marguerite and Cineraria is **non-existent** ; there are **no stamens**. Hence the ray florets in these plants are female only or **pistillate**. Clearly the chief function of the ray florets is to make the whole capitulum conspicuous to insects, and the loss of the stamens seems to be correlated with the great development of the ligulae of the corollas.

(2) **A Disk Floret**.—Remove a disk floret from its attachment to the disk and examine it in detail.

Observe the **inferior ovary** and the **calyx** at its top. The calyx in the **Marguerite** is almost absent, being represented by a yellowish-green collar at the top of the ovary ; while, in the **Cineraria**, the calyx is made up of fine silky hairs, known collectively as the **pappus** which persists and serves to disseminate the fruit (the ripened ovary with its single seed).

Notice that the **corolla** of a disk flower is entirely tubular and actinomorphic (regular), thus differing greatly from that of a ray floret. The **corolla-tube** is cylindrical below and expanded like a bell above, and it terminates in **five** segments or corolla lobes. Count the corolla lobes. Evidently the corolla is pentamerous and gamopetalous.

The **androecium** consists of **five stamens**. Their filaments are five separate slender threads which spring from the base of the upper bell-like expansion of the corolla-tube. The **anthers** are **united** together **laterally** to form a **tube** around the style. The pollen is liberated by the anthers into the anther-tube and then it is pushed out of the tube by the style as this grows in length. **Find the anther-tube** made by the five united anthers ; it projects upwards in the middle of the bell-shaped part of the corolla.

The **gynaecium** of a disk floret, like that of a ray floret, consists of an **inferior ovary** (already seen), a slender **style**, and **two stigmas**. In **older** disk florets—those toward the outside of the disk—the style may be seen projecting through the anther-tube and bearing its two stigmas which diverge from one another. But in younger disk florets—those nearer the middle of the disk—the style with

its two stigmas may still be hidden in the anther-tube, and then these structures are invisible.

Pollination and the Floral Mechanism.—The ray florets of the capitulum through their conspicuous strap-shaped corollas serve to attract bees, butterflies, and other insects to the capitulum as a whole. Nectar is secreted at the base of the tube of each corolla and insects, in passing from one floret to another to suck up the nectar, bring about cross-pollination.

The disk florets are all **proterandrous**, i.e. the anthers dehisce and shed their pollen before the stigmas have diverged from one another and are ready to receive pollen. In each disk floret the succession of events is as follows : The style and its two stigmas form a rod enclosed in the anther-tube. The anthers liberate their pollen into the anther-tube. The rod-like style elongates and pushes the pollen out of the anther-tube. The pollen thus heaped up at the mouth of the anther-tube is carried off by insects. Then the style emerges from the anther-tube and the two stigmas at its end diverge from one another and become receptive on their hairy inner sides. Thus **each disk floret**, functionally, is **first male** and then **female**.

In a developing capitulum, at the right stage, one can make out three zones of disk florets : (1) an **inner zone** of florets, occupying **the centre of the disk**, which are very young and have **not yet opened** ; (2) a **median zone** of open florets, in which there are **masses of pollen** at the mouths of the anther-tubes but in which **no stigmas** are as yet visible ; and (3) an **outer zone** of florets, the oldest of all, which have **lost their pollen** and which display their **divergent stigmas**. An insect moving about on a capitulum necessarily carries pollen from the more central younger flowers to the stigmas of the outer older flowers, thus bringing about cross-pollination. When an insect visits the capitula of several plants in succession, it may bring about cross-pollination between the flowers of different plants.

The Violet as a Type of the Violaceae.

The floral formula for a Violet is

Ca.5, Co.5, A.5, G.3.

In the Violet supplied make out the five separate green **sepals** and the five separate violet **petals**. Notice that the lower petal is extended backwards into a **spur**.

The **five stamens** have very short filaments and are close together around the ovary. Notice that from the two front stamens **two rod-like processes** extend backwards into the spur of the anterior petal. These processes are **nectaries** and they excrete nectar which accumulates in the spur.

The **gynaeceum** consists of a tricarpeillary, unilocular ovary, a style, and a hooked stigma. The ovules in the ovary are numerous and situated on three parietal placentae.

Bees visit the flowers and bring about their cross-pollination.

The Snapdragon as a Type of the Scrophulariaceae.

The floral formula for the Snapdragon is :

Ca.(5), Co.(5), A.4, G.2.

In the Snapdragon supplied make out the five green **sepals** united at their base and then the corolla. The corolla is **bilabiate**, i.e. has two lips. The upper lip consists of **two** united **petals**, and the lower lip of **three** united **petals**. Pull down the lower lip and then let it go ; it will snap back into its old position. Hence the name Snapdragon. Notice that the **stamens** are only **four** in number and that they are in two pairs—a longer pair and a shorter pair. The fifth or back stamen is missing. If present it would be in the way of the style. The ovary has only two chambers but each one contains many ovules. The Snapdragon is a good example of a zygomorphic flower. Humble bees force open the flower's "strong-box" and effect cross-pollination.

CHAPTER XXIV

FRUITS AND THEIR ENCLOSED SEEDS

The following fruits will be supplied :

- (1) A fresh green legume of the Edible Pea ;
- (2) A woody legume of the Pea-nut ;
- (3) A Hazel nut, intact ; and another Hazel nut with a fractured pericarp ;
- (4) A Walnut ;
- (5) A Sunflower fruit which has been soaked in water to soften it ;
- (6) A Corn fruit which has been soaked in water to soften it ;

- (7) A fresh berry of the Large Commercial Cranberry ;
- (8) A Date-palm fruit ;
- (9) A Tomato fruit ;
- (10) An Orange fruit ;
- (11) A Strawberry fruit ;
- (12) An Apple fruit.

The actual practical work begins with the Section called *Examination of Certain Fruits* (p. 244). The preliminary matter (pp. 237-244) should be read over carefully before the class begins.

THE FRUIT OF ANGIOSPERMS

Origin of Fruit.—When the ovules in the ovary of a flower have been fertilised by the male nuclei brought to their egg-cells by the pollen-tubes, new growth takes place in: (1) the **ovules**, which develop into seeds; in (2) the **ovary-wall**, which now enlarges in size, thickens, and becomes altered in texture, thus developing into a **pericarp** (fruit-wall); and, sometimes, in (3) one or more **neighbouring parts of the ovary**, e.g. the **receptacle**, which may become fleshy or otherwise altered. The product of all this secondary growth is known as the **fruit**.

In the wide sense, therefore, the **fruit** of an Angiosperm (i.e. of a Monocotyledon or a Dicotyledon), is the **entire result of secondary growth** induced in the **ovary** and **neighbouring parts** of the flower by the stimulus of fertilisation.

The enlarged ovary-wall of a fruit is always known as the **pericarp**. Most fruits consist simply of the **pericarp** and its enclosed **seeds**.

Kinds of Fruit.

Fruits developed simply from a ripened ovary (i.e. most fruits) have been known as **true fruits**, whereas fruits which are developed from the ripened ovary plus the swollen receptacle, etc., have been known as **false fruits** or **pseudocarps**. This distinction, although not very fundamental, is useful for classificatory purposes.

Fruits developed from the ripened ovary only, i.e. the so-called **true fruits**, may be divided into **simple fruits** and **aggregate fruits**.

Simple and aggregate fruits are both developed from a **single flower**. A **simple fruit** is formed from a gynaeceum which is monocarpellary (Pea, Cherry) or syncarpous (Tulip, Daffodil, Tomato, Orange), whereas an **aggregate fruit** is formed from a

gynaeceum which is apocarpous, i.e. one in which the carpels are many and separate from one another (Buttercup, Prairie Crocus, Raspberry).

Simple fruits may be subdivided into **dry** and **succulent**. In **dry fruits** (Sunflower, Hazel, Poppy) the pericarp is dry and firm, whereas in **succulent fruits** (Tomato, Orange, Date, Plum) the pericarp is more or less fleshy and juicy.

Simple fruits may be further subdivided into **indehiscent** and **dehiscent**. An **indehiscent fruit** is one in which the pericarp **does not split open** and liberate the seeds (Sunflower, Hazel, Cherry, Orange), whereas a **dehiscent fruit** is one in which the pericarp does split open to liberate the seeds (Pea, Larkspur, Willow, Poppy).

Certain polycarpellary syncarpous many-seeded fruits are known as **schizocarps** or **splitting fruits**. When ripe their pericarp splits up into a number of one-seeded parts or **mericarps**. Each mericarp consists of a piece of pericarp enclosing a single seed (Hollyhock, Manitoba Maple).

There are various ways of classifying fruits. The following classification may prove convenient for reference in the practical work about to be undertaken :

Classification of Fruits.

True Fruits, developed from an ovary only.

I. **Simple**, developed from a monocarpellary or a syncarpous ovary.

A. **Dry**, pericarp dry and hard.

(1) **Indehiscent**, pericarp not splitting open.

(a) **One-seeded**.

Achene ovary superior, pericarp free from testa, e.g. Buttercup, Prairie Crocus.

Caryopsis ovary superior, pericarp fused with testa, e.g. Corn, Wheat, and all Grasses (grains).

Cypsela ovary inferior, pericarp free from testa, e.g. Sunflower, Daisy, and all Compositae.

Samara pericarp winged, e.g. Ash, Elm.

Nut..... pericarp hard and woody,
forming a shell, e.g. Hazel,
Sweet Chestnut, Oak.

(b) **Many-seeded**, known as **schizocarps** or **splitting fruits**.

Double Samara ... splitting into two winged
pericarps or samaras, e.g.
Maple.

Cremocarp ovary inferior, pericarp split-
ting into two mericarps,
e.g. the Umbelliferae.

Carcerulus ovary superior, bicarpellary,
quadrilocular owing to
presence of two false septa ;
pericarp splits into four
nutlets ; the characteristic
fruit of Labiatae and
Boraginaceae, e.g. Forget-
me-not, Mint, Borage.

Lomentum ovary elongated, pericarp
splits transversely into
mericarps, e.g. Radish,
Hedysarum.

(2) **Dehiscent**, pericarp splits open to liberate the seeds.

(a) **Monocarpellary**, consisting of a single carpel.

Follicle dehisces along the ventral
suture only, e.g. Larkspur,
Aconite, each flower having
several follicles.

Legume (pod) dehisces along both
dorsal and ventral sutures,
e.g. Pea, Bean, and other
Leguminosae.

(b) **Bicarpellary** or **polycarpellary**, consisting of two or
more carpels.

Silique bicarpellary, with a false
septum dividing the ovary
into two loculi, placentae
parietal, pericarp forms two
valves which separate from

the septum and placentae,
fruit much elongated, e.g.
Wallflower, Mustard, Stock.

Silicula like a siliqua but broad
instead of long, e.g. Shep-
herd's Purse, Honesty.

Capsule formed from a bicarpellary,
tricarpellary, or polycar-
pellary, syncarpous ovary.

Dehiscing by pores ... e.g. Poppy, Campanula,
Snapdragon.

Dehiscing by teeth ... e.g. Pink, Primrose.

Dehiscing transversely e.g. Plantain, Pim-
pernel, Henbane.

Dehiscing longitudinally :

and loculicidally ... e.g. Tulip, Lily.

and septicidally..... e.g. Foxglove, Colchi-
cum.

and septifragally ... e.g. Rhododendron.

B. Succulent, pericarp fleshy and juicy.

(1) **Indehiscent**, pericarp not splitting open.

Drupe..... (stone-fruit), pericarp differ-
entiated into an **epicarp** or
outer skin, a **mesocarp** or
middle fleshy or sometimes
fibrous region, and an **endo-
carp**, the "stone" which
encloses and protects the
seed :

Simple, with only one "stone."

Monocarpellary e.g. Cherry, Plum,
Peach.

Bicarpellary e.g. Olive.

Tricarpellary e.g. Coconut (mesocarp
fibrous).

Compound, with several "stones" enclosed in
the mesocarp :

Polycarpellary e.g. Holly, Dogwood,
Elder, the fruits of
which are popularly
called "berries."

Berry pericarp more or less pulpy,
seeds embedded in the pulp,
no "stone" present, de-
veloped from a superior or
an inferior ovary.

Superior e.g. Grape, Date, To-
mato.

Inferior e.g. Cranberry, Blue-
berry, Gooseberry,
Currant, Banana,
Cucumber. The
Banana berry is
seedless owing to
cultivation.

Hesperidium developed from a multilocular
superior ovary with axillary
placentation. It is very
similar to a berry, from
which it differs in having
a pericarp which is
leathery instead of pulpy.
The pulp of the fruit
consists of internal emer-
gences which grow into
and fill the loculi, e.g.
Orange, Lemon, Grape-
fruit.

(2) **Dehiscent**, pericarp splits open.

Succulent Capsule e.g. Horse Chestnut.

Dehiscent Drupe .. e.g. Walnut. The thin fleshy
epicarp peels off and
allows the stony endocarp
with its enclosed seed to
escape.

Dehiscent Berry .. e.g. Nuphar, the Yellow
Water Lily.

**Dehiscent Schizo-
carp or Regma** e.g. Geranium. Here the
pericarp splits into **cocci**,
each of which dehisces and
liberates its single seed.

II. **Aggregate Fruits**, developed from an apocarpous gynaeceum (carpels separate, not fused). The aggregation of simple fruitlets formed by a single flower is called an **etaerio**.

An **etaerio of achenes** e.g. Buttercup, Prairie Crocus, Clematis.

An **etaerio of follicles** e.g. Larkspur, Monkshood, Spiraea.

An **etaerio of drupes**..... e.g. Raspberry, Blackberry.
or **drupels**

Pseudocarps or False Fruits.

(1) **The Fig**.—The Fig fruit is formed from an inflorescence. The inflorescence is a hollow pear-shaped capitulum. The top of the peduncle has become hollowed out so that its wall encloses a cavity. The **flowers** are developed inside the cavity in large numbers. The **ovaries** of the tiny female flowers develop into fruits like **achenes**. These little rounded yellow fruitlets are usually referred to by fig-eaters as “seeds.” The fleshy part of a Fig fruit is nothing more than a hollowed and thickened terminal part of the peduncle of an inflorescence. The Fig fruit is a composite fruit in that it encloses many little fruitlets within its peduncular receptacle.

(2) **The Pine-apple**.—This remarkable fruit is formed from a spike, i.e. from an inflorescence consisting of a peduncle bearing flowers which have no pedicels. The flowers, through cultivation, have become sterile and seedless. The Pine-apple fruit is formed by the **peduncle**, its **bracts**, and the numerous **flowers** all becoming fleshy and thus forming one fleshy, juicy mass. Each of the diamond-shaped areas on the outside of a fruit corresponds to a flower. At the top of the fruit the peduncle bears a crown of leaves. This can be cut off and planted as a cutting. It takes root and, in course of time, may form another Pine-apple fruit. A fleshy collective fruit, like that of the Pine-apple, Fig, or Mulberry, is sometimes called a **sorosis**.

(3) **The Mulberry**.—This again is a fleshy collective fruit or **sorosis** developed from an inflorescence. The inflorescence, as in the Pine-apple, is a spike. All the flowers in the female spike develop **fleshy perianths**, each perianth enveloping a single true fruit (the ripened ovary). A single mulberry, therefore, is made up of a number of fleshy perianths enclosing as many true fruits.

(4) **The Strobile or Cone**.—This is a scaly multiple fruit. The

name is applied to the fruit of the **Hop** where the large and thin scales are bracts of a female inflorescence. The true fruits or ripened ovaries occur in pairs above the bracts and are dry, indehiscent, and one-seeded.

The term **cone** (or strobile) is especially applied to the fruit of Pines, the Tamarack, the Balsam Fir, etc., i.e. to **Coniferae**. However, the cone of a Pine, etc., is the product of a **single** female flower and not of many flowers. The naked seeds in such a cone are borne on the upper face of flattened fructiferous scales which become much thickened and woody.

(5) **The Strawberry**.—The fruit of the Strawberry is developed from a **single** flower. The flower has an **apocarpous** gynaeceum, i.e. the carpels are separate from one another and not joined laterally. Each carpel develops into a tiny yellowish achene. Underneath the numerous achenes the **receptacle** becomes much dilated and forms a red sweet succulent mass of tissue ; so that, when a strawberry is ripe, the achenes can be seen well separated from one another all over the conical or globular receptacle. It is therefore the receptacle, and not the ripened ovaries of the achenes, which makes a Strawberry fruit succulent.

(6) **The Rose Fruit**.—The fruit of a Rose, often called a **hip**, is developed from a single flower. The gynaeceum, as in the Strawberry, is **apocarpous**. The receptacle is hollowed out like a jug ; and the separate carpels are inserted on the inner side of the receptacle and are therefore hidden from view. The wall of the hollow receptacle turns from green to red and becomes fleshy. The ovaries of the carpels on ripening become **leathery** or **bony achenes**. In the Rose fruit, therefore, it is the **receptacle** that provides the succulent tissue.

(7) **The Apple or Pear Fruit**.—This is called a **pome**. In the flower of an Apple or Pear tree there are five imperfectly fused carpels enclosed in a hollow cup-like receptacle. As development proceeds, the five ovaries of the five carpels become cartilaginous, while the receptacle which envelops them becomes fleshy. It is therefore the **receptacle** of an Apple or Pear fruit which provides the succulent matter.

(8) **The Aromatic Wintergreen**.—This plant, *Gaultheria procumbens*, has fruits which are popularly called berries ; but each berry consists of a capsule (developed from the ovary) enclosed from below by a greatly enlarged succulent **calyx**. This fleshy calyx contains oil of wintergreen and has a spiey-aromatic flavour.

Dispersal of Seeds and Fruits.

In studying fruits, it should be borne in mind that the peculiar form and consistency of each kind of fruit are correlated with the manner in which the seeds or the fruits are dispersed, i.e. that, in fruits, structure and function are closely correlated.

Very small seeds, e.g. those of Orchids, winged seeds, winged fruits, plumed seeds, and plumed fruits are carried off by the **wind**; whereas hooked fruits and succulent fruits are dispersed by **animals**. The succulent parts of succulent fruits are eaten with relish by various animals, e.g. birds, pigs, etc., and, as a rule, the seeds are swallowed but not digested, so that the seeds eventually are evacuated from the animal's body uninjured in the excreta. Certain fruits, e.g. Touch-me-not and the Squirting Cucumber, are **explosive** and shoot their seeds to a distance of several feet.

EXAMINATION OF CERTAIN FRUITS

(1) **The Legume or Pod of a Pea.**—Examine the Pea fruit provided. It has developed from an ovary like that of the Sweet Pea studied in the last lesson. Notice the **pedicel**, the **receptacle**, the persistent **calyx**, and perhaps the remains of the **style** at the free end of the legume.

The **pericarp** (swollen ovary wall), although still green, is becoming leathery. Using both hands and beginning at the stylar end of the fruit, pull the two halves of the pericarp apart, so as to **split the pod to its base**. Evidently the legume **dehisces by two sutures**—a **dorsal** (under) suture and a **ventral** (upper) suture. Notice that the seeds (like the ovules from which they have developed) are attached to the placenta in a double line along the top of the fruit.

In your first practical lesson you made a drawing of a Pea pod. Turn to it now and see that the pericarp was labelled as such and that the attachment of the seeds to the placenta was accurately represented.

A **legume** is a dry, monocarpellary, dehiscent fruit which dehisces along both its dorsal and ventral sutures. It is characteristic of the fruit of the Leguminosae. A reference to the Classification of Fruits already set out in this Chapter will show that a legume may be further described as a **true** fruit and as a **simple** fruit.

(2) **The Woody Legume of a Pea-nut Plant.**—Examine the Pea-nut fruit provided. The flower which bore it was pollinated and its ovules fertilised in the air in the usual way. When the ovules had been fertilised, the pedicel of the flower became positively geotropic, it elongated and grew down into the soil, and then it pushed the ripening ovary into the soil. The fruit then ripened underground. To obtain pea-nuts, one has to dig them up.

Notice that the pericarp of the Pea-nut legume is more woody than leathery. **Press the pericarp** above and below and you will find that, like a Pea-pod, it splits or **dehisces along two sutures** (dorsal and ventral) into two halves (valves). Observe the **seeds**. You will remember that, in your second practical lesson, you found that each seed consists of a thin brown testa enclosing an embryo made up of a radicle and plumule and two large fleshy cotyledons. Turn to your old drawing and see that the pericarp was labelled as such.

(3) **The Hazel Nut.**—In a nut the pericarp is hard and woody, forming a **shell**. Examine the Hazel nut (filbert) provided. The hard brown external shell is the pericarp. The point at the top was once prolonged into two long styles, each ending in a red feathery stigma. The broad scar below the nut shows where the nut was once attached to a twig. **Shake the nut** near your ear. You may find that the seed inside is loose and can be rattled against the pericarp.

Take out the **seed** from a Hazel nut with a fractured pericarp. Notice that the seed is covered by a thick brown testa and is **pointed** at one end. The axial mass of the embryo, i.e. the radicle and plumule, is at this point. Cut open the seed and observe the embryo. Find its two large fleshy cotyledons, its radicle, and its plumule.

(4) **The Walnut Fruit.**—The Walnut fruit is a **dehiscent drupe** (stone-fruit). Its pericarp becomes differentiated into a fibrous-fleshy **epicarp** and a stony **endocarp**. The epicarp splits open and liberates the seed enclosed in the stony endocarp. The walnut provided, therefore, is a fruit consisting of only **part of the pericarp** and **one seed**. Walnuts are pickled when they are very young before the pericarp has formed its stony endocarp, and it is the pericarp that is eaten.

The stony endocarp of the walnut readily splits into two halves or valves. **Split open your specimen** with a pen-knife. Observe that the endocarp enclosed a single cavity partly divided

into four by incomplete septa which are inward extensions of the endocarp. Held within the cavity is a **single seed**. The seed consists of a thin brown testa and an enclosed embryo. The embryo consists of two large **fleshy and contorted cotyledons**, a small **radicle**, and a small **plumule**.

One end of the stony endocarp is **pointed**. The radicle in the embryo points toward the point. When you have opened the endocarp, try and make out the parts of the embryo enclosed by the testa. Look for the radicle at the point just mentioned.

(5) **The Sunflower Fruit**.—This kind of fruit is known as a **cypsela**. A cypsela is a dry, indehiscent, one-seeded fruit which is developed from an **inferior** ovary. It is the characteristic fruit of the **Compositae**. Many cypselas, e.g. Dandelion, Sow Thistle, Cineraria, are crowned above by a **hairy pappus** which assists in their dissemination by the wind. However, in a Sunflower fruit there is no pappus present.

The Sunflower fruit supplied has been soaked in water to soften it. Its **upper** end is the **larger** end. Remove the horny pericarp and find the seed enclosed. Notice that the seed is covered by an extremely thin and papery testa and is pointed at its lower end. Within the testa is an embryo with two large fleshy cotyledons, a radicle, and a plumule. Try to verify these statements.

(6) **The Corn Fruit**.—This kind of fruit, in our cereals popularly known as a **grain**, has received the technical name **caryopsis**. A caryopsis is a dry, indehiscent, one-seeded fruit in which the **pericarp and the testa are fused together**, so that they form a single thin tough membrane. The caryopsis is the characteristic fruit of the Gramineae, i.e. of all grasses including cereals.

It is impossible to take the seed out of the pericarp easily as in a Hazel nut or Sunflower fruit. Take the outer membrane off the Corn fruit provided. The **membrane** consists of **pericarp plus testa**. Hence, when this membrane has been removed, there is left behind just an embryo and the endosperm, i.e. something less than the whole seed.

(7) **The Cranberry Fruit**.—The Cranberry fruit is a **berry**. A **berry** is a succulent fruit in which the whole of the pericarp is more or less **pulpy**.

The berry supplied belongs to *Vaccinium macrocarpon*, the Large or American Cranberry. The berries come from the United States and are on sale in shops in Winnipeg throughout

the winter. Our Blueberries (*V. pennsylvanicum* and *V. canadense*) have fruits which in structure resemble Cranberries, although they differ in colour, size, and flavour.

A cranberry is developed from an inferior ovary and may therefore be called an **inferior berry**. Notice the **calyx**, made up of **four sepals**, still left on the top of the ripened ovary.

Cut the cranberry supplied transversely into two halves, and examine its interior. The outer firm skin of the pericarp may be called the **epicarp**, and the rest of the pericarp, i.e. the soft red pulpy tissue including the lateral walls of the loculi, may be called the **endocarp**. Notice the **four chambers** or loculi (the gynaecium was made up of four carpels and was syncarpous) and the tiny **seeds** attached to the central axillary column (the placentation of the ovules was axillary).

(8) **A Date Fruit**.—The Date fruit comes from the Date Palm, *Phoenix dactylifera*, and is a berry. As already explained, a berry is a succulent fruit in which the pericarp is more or less pulpy. A Date berry is developed from a superior ovary and therefore may be classified as a **superior berry**. Sometimes the hard dry **calyx** is left at one end of the fruit, but more frequently it has been removed.

Cut the Date berry longitudinally into two halves so as to expose the stony seed.

Notice that the **pericarp** (the ripened ovary wall) is made up of three layers: (1) an **epicarp** or smooth outer skin; (2) a **mesocarp**, which is succulent; and (3) a papery **endocarp**. The papery endocarp will be found around the seed.

The “stone” of the Date fruit is simply a **horny seed**. The seed was studied in a previous lesson. You will remember that it consists of an external very thin brown **testa**, a minute median **embryo**, and a large horny **endosperm**, the endosperm having its reserve carbonaceous food material stored up in the form of hemi-cellulose in its thickened cell-walls. Remove the seed from the pericarp and find on its rounded side the depression which marks the position of the embryo beneath the testa.

(9) **The Tomato Fruit**.—The Tomato plant, *Lycopersicon esculentum*, has a fruit which is a typical berry. Since the berry is developed from a superior ovary, it may be called a **superior berry**.

Examine the Tomato berry supplied. Notice its stalk or **pedicel end** and the scar at its apex which marks the **position**

of the style before this disappeared. Notice also the smoothness and brilliant red colour of the epicarp. The red colour is due to the presence of a hydrocarbon pigment known as **lycopersicin**. This pigment, which also occurs in Red Pepper (*Capsicum annuum*), has the same formula as carotin, $C_{40}H_{56}$. Yellow Tomatoes have carotin as their skin-pigment instead of lycopersicin.

If not already done, cut the fruit through **transversely** and examine the surfaces of section.

Notice that the interior of the berry is divided up into several chambers or **loculi**. In the wild ancestors of our cultivated plants, which were of South American origin, there were only two loculi in the ovary, but the number has been increased by cultivation. The horticultural forms of the Tomato have been produced by crossing wild and cultivated species and varieties.

Notice the **epicarp** (outer skin) and the **endocarp**, i.e. the rest of the pericarp including the **dissepiments** (lateral walls of the loculi), the enormously enlarged **axillary column**, and the massive **placentae** stretching out from the axillary column into the loculi and bearing the **seeds**.

By cultivation, crossing, and selection, the Tomato berry has been greatly improved for the use of man. This improvement consists largely in the increase in size of the fruit as a whole, an increase in the solidity and amount of the pulpy parts of the fruit, and an increase in the smoothness of the surface.

Make a drawing of a Tomato berry in transverse section. Indicate the **epicarp**, **endocarp** and its **dissepiments**, the **axillary column**, the **placentae**, and the **seeds**. Make the drawing natural size.

(10) **An Orange Fruit.**—The Orange tree, *Citrus Aurantium*, has a fruit which is known as a **hesperidium**. A hesperidium differs but little from a berry; for it is a berry in which the external wall of the pericarp is leathery instead of pulpy, the pulp being provided by emergences which grow into the loculi and envelop the seeds. The hesperidium is the characteristic fruit of a section of the family *Rutaceae* which includes not only the Orange (*Citrus Aurantium*), but also the Lemon (*C. medica* var. *Limonum*), and the Grape-fruit (*C. decumana*). The Grape-fruit is an independent species of *Citrus*, and not a hybrid between the Orange and Lemon as is often erroneously supposed.

The hesperidium of the Orange is developed from a **superior**

ovary. Like the Tomato fruit just examined, the orange is a **multilocular, superior fruit** with **axillary placentation** and **numerous seeds**.

Examine the orange externally. Notice the basal greenish **calyx**, if it is still attached, and the **apical scar** which marks the place where the style was. Notice, further, **drops of oil** in the pericarp, which can be seen as tiny watery-looking round spots scattered in great numbers in the pericarp just below its surface. The oil drops are contained within the cavities of oil glands.

If not already done, cut the orange **transversely** into two halves, and study the surfaces of section.

Notice that the pericarp consists externally of what is commonly called the **rind**, made up of (1) an outer red glandular skin or **epicarp** (look for the glands) and (2) a thick whitish rather leathery **endocarp** (most of the "peel"). The endocarp is continued to the centre of the fruit in the form of dissepiments which meet and form an **axillary column**. Attached to this column are the **seeds**, often called **pips**. Evidently the placentation of the ovules was axillary.

Examine the **pulp** carefully. With the help of a needle **lift up some of the pulp** and notice that it is made up of much swollen, stalked, more or less **spindle-shaped outgrowths** which spring from the walls of each loculus, are directed towards the axillary column, crowd against one another, and fill up each locular cavity. These outgrowths are known as **emergences** and not as hairs, because they originate not from a single cell but from a mass of cells. They each contain a vascular bundle which comes to them through the fruit-stalk and rind. The **juice of the orange** is simply cell-sap contained within the large cells of the hair-like emergences. The emergences by growing out from the sides of the loculi into the locular cavities come to envelop the seeds, thus increasing the chances that the seeds will be swallowed with the pulp by a frugivorous animal.

The **seeds** (pips) have a **mucilaginous testa** and are so slippery that they readily **slide down the throat of an animal eating the pulp**. There can be no doubt that the slipperiness of the seeds, and perhaps their peculiar shape, is correlated with the mode of seed dispersal.

A final point about orange seeds may be mentioned. It is that the seeds exhibit **polyembryony**, i.e. the enclosure within the testa of more than one embryo (several embryos in one

seed). Often there are two or three embryos in the same seed and there may be as many as ten or even thirteen. One of these embryos arises in the usual way from a fertilised egg-cell, while all the other embryos arise by "budding" as outgrowths of the nucellus which surrounds the embryo-sac. Polyembryony complicates the breeding of new Orange varieties. Remove the testa from a seed and try to find the embryos which it encloses.

Make a drawing of a transverse section of an Orange fruit. Show the glandular **epicarp**, the tough whitish **endocarp**, the **dissepiments** of the loculi, the **axillary column**, the juicy **emergences** projecting into the locular cavities, and a few **seeds** attached to the axillary column.

(11) **A Strawberry Fruit**.—The fruit of the Strawberry is classified as a **pseudocarp** or false fruit because it is formed from something more than the ripened ovary or ovaries. It may be described as a **collection** (etaerio) of **achenes** borne on a much swollen, pulpy **receptacle**.

Examine the Strawberry fruit provided. Notice the pedicel below. Its top, the **receptacle**, is (1) flattened out to form a small **green saucer-like expansion** which bears the calyx (notice the persistent green sepals), the corolla (the petals have disappeared), and the androecium (remains of the stamens may still be attached), and (2) enormously expanded in the centre to form a **conical or globular juicy red mass of tissue**. On this succulent part of the receptacle and scattered in considerable numbers over its surface are borne the **achenes**, little hard yellowish-green structures. Examine some individual achenes. Notice that **each achene** consists of a yellowish-green **ripened ovary** (it contains one seed like a Sunflower fruit and the ovary wall is the pericarp), a black hair-like **style**, and a little black terminal **stigma**.

The flower of the Strawberry is **perigynous**, the sepals, petals, and stamens being inserted on the receptacle, **not under** the gynaecium but **around** it on the saucer-like expansion of the receptacle. The gynaecium of the flower is **apocarpous**, i.e. its carpels are not joined together laterally but are quite separate from one another. Each carpel, therefore, has its own ovary, style, and stigma. In each ovary is a single ovule and this, after fertilisation, becomes a seed enclosed in the ripened ovary-wall or pericarp. The carpels are borne on the terminal swollen part of the receptacle free from the saucer-like part.

Cut the Strawberry fruit vertically downwards with a knife and notice the vascular bundles which appear as whitish streaks passing upwards and outwards to the achenes.

(12) **The Apple Fruit.**—Here, again, we have a fruit which may be classified as a **pseudocarp** or false fruit because it is formed from something more than the ripened ovary or ovaries. The Apple fruit is known technically as a **pome** and consists of a **fleshy receptacle** enclosing the five ripened ovaries of **five carpels**, the inner walls of which are cartilaginous. The carpels are only partially fused together in the centre of the fruit. The pome is the characteristic fruit of the Apple, Pear, Medlar, etc., i.e. of the Pomeae, a tribe of the family known as the **Rosaceae**.

Examine the apple provided externally. Notice the **pedicel** or **fruit-stalk** with a **scar** at its end showing where it separated from the twig which bore it. An **absciss-layer**, a special layer of thin tissue, is developed across the stalk at the point where it breaks, and it is this which enables the apple fruit to fall when it is ripe. By falling to the ground, an apple comes to be in a position where it may be found by ground animals, e.g. pigs, rodents, etc., which may aid in the dispersal of the seeds. The upper end of the stalk passes into a depression of the fleshy receptacle. Pear fruits lack this depression.

Notice that at the apex of the apple, seated on the very top of the fleshy receptacle, are the withered **sepals** of the calyx and perhaps the withered remains of the **stamens**. The petals fell away and have left no trace of themselves.

The skin of the apple is highly cuticularised and is very resistant to the passage of water, so that an intact apple dries up very slowly. From the commercial point of view this is a good keeping-quality.

If not already done, cut open the apple **transversely**. Then notice the five ovaries of the five carpels in the centre (each containing one or two brown seeds) and the massive fleshy receptacle surrounding them. The carpels touch one another at the centre of the fruit, but otherwise are separated. The inner wall of the pericarp of each ripened ovary is obviously **cartilaginous**. This cartilaginous wall looks like the whole of the pericarp, but investigations have shown that it must be regarded as an **endocarp** only, for outside it, developed from the ovary wall, is a little mesocarp and epicarp which are both fleshy and which have fused with the massive fleshy receptacle so that the junction

line can only faintly be traced in the ripe fruit or not at all. In our cross-section, then, what we clearly see is a **massive fleshy receptacle** enclosing **five ripened ovaries** (true fruits) **with cartilaginous pericarps**.

In each of the five ovaries are at first **two ovules**, so that it is possible for two seeds to develop in each carpel. An apple, therefore, may contain as many as **ten seeds**. Sometimes there are less than this number produced owing to some of the ovules not having been fertilised.

When the Apple flower was developing, the top of the pedicel or **receptacle** grew up around the five carpels and enveloped them and bore at its apex the sepals, petals, and stamens. Each carpel had an ovary containing two ovules, a style, and a stigma, and the five stigmas protruded upwards between the sepals, petals, and stamens at the apex of the flower. After fertilisation of the ovules, the inner walls of the five ovaries became cartilaginous to form what is popularly called the "core" of the apple, while the receptacle thickened and became solid-fleshy to form the succulent part of the apple.

In the cross-section of the apple fruit notice the **ring of ten vascular bundles** in the fleshy receptacle. Within this ring is the pith of the receptacle and outside it is the **cortex** of the receptacle.

As soon as the apple has been cut open, it usually **begins to turn brown** at the cut surface. Is your apple exhibiting this phenomenon? The brown colouration is due to the action of an oxidising enzyme or **oxidase**. Where the cells have been injured, the oxidase causes the oxygen of the air to unite with a colourless organic substance present in the cells, with the result that there is produced a new compound which is brown. It is this brown compound which gives the brown colour to the eye.

Make a drawing of a transverse section of the Apple fruit. Indicate the **five ripened ovaries** with their **cartilaginous pericarps**, the **seeds**, and the **fleshy receptacle** with its **ten vascular bundles**, its **pith** and its **cortex**. Make the drawing natural size.

APPENDIX

ON THE CLASSIFICATION OF PLANTS

THE PLANT KINGDOM

CRYPTOGAMIA (Sporophyta) Flowerless
Plants.

I. Thallophyta—

1. Bacteria *e.g.* Hay bacillus.
Bacterium
radicicola.
Bacillus
anthracis.
2. Algae *e.g.* Protococcus.
Closterium.
Spirogyra.
Sea - weeds,
e.g. Fucus.
3. Fungi *e.g.* Yeast.
Mucor.
Rhizopus.
Penicillium.
Mushroom,
etc.
4. Lichens *e.g.* Xanthoria.
Cladonia.

II. Bryophyta—

1. Hepaticae Liverworts *e.g.* Marchantia.
2. Musci Mosses *e.g.* Funaria.
Sphagnum.

III. Pteridophyta—

1. Filicales Ferns *e.g.* Aspidium.
2. Equisetales ... Horsetails *e.g.* Equisetum.
3. Lycopodiales... Club-mosses *e.g.* Lycopodium.

PHANEROGAMIA (Spermaphyta) Flowering
Plants.

I. Gymnospermae—

1. Coniferae *e.g.* Pine, Spruce,
Balsam Fir.

II. Angiospermae—

1. Monocotyledones *e.g.* Lily, Wheat,
Palms.
2. Dicotyledones *e.g.* Rose, Pea,
Oak.

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